Microbiology

Disrupting folate metabolism reduces the capacity of bacteria in exponential growth to develop persisters to antibiotics

--Manuscript Draft--

Manuscript Number:	MIC-D-18-00063R1
Full Title:	Disrupting folate metabolism reduces the capacity of bacteria in exponential growth to develop persisters to antibiotics
Article Type:	Research Article
Section/Category:	Physiology and metabolism
Corresponding Author:	J. ENRIQUE SALCEDO-SORA, M.D., Ph.D. Liverpool Hope University Liverpool, Merseyside UNITED KINGDOM
First Author:	Jasmine Morgan, BSc
Order of Authors:	Jasmine Morgan, BSc
	Matthew Smith
	Mark T Mc Auley, PhD
	J. ENRIQUE SALCEDO-SORA, M.D., Ph.D.
Abstract:	Bacteria can survive high doses of antibiotics through stochastic phenotypic diversification. We present initial evidence that folate metabolism could be involved with the formation of persisters. The aberrant expression of the folate enzyme gene fau seems to reduce the incidence of persisters to antibiotics. Folate impaired bacteria had a lower generation rate for persisters to both antibiotics ampicillin and ofloxacin. Persister bacteria were detectable from the outset of the exponential growth phase in the complex media. Gene expression analyses showed tentatively distinctive profiles in exponential growth at times when bacteria persisters were observed. Levels of persisters were assessed in bacteria with altered, genetically and pharmacologically, folate metabolism. This work shows that by disrupting folate biosynthesis and usage, bacterial tolerance to antibiotics seems to be diminished. Based on these findings there is a possibility that bacteriostatic antibiotics such as antifolates could have a role to play in clinical settings where the incidence of antibiotic persisters seem to drive recalcitrant infections.

Disrupting folate metabolism reduces the capacity of bacteria in exponential growth to develop persisters to antibiotics

Jasmine Morgan, Morgajas@edgehill.ac.uk, Department of Biology, Edge Hill University, St. Helens Road, Ormskirk, Lancashire, L39 4QP, UK

Matthew Smith, smithm2@hope.ac.uk, School of Health Sciences, Liverpool Hope University, Hope Park, L16 9JD, Liverpool, UK

Mark T. Mc Auley, m.mcauley@chester.ac.uk, Chemical Engineering Department, University of Chester, Thronton Science Park, CH2 4NU, Chester, UK

J. Enrique Salcedo-Sora (Corresponding Author), salcede@hope.ac.uk, School of Health Sciences, Liverpool Hope University, Hope Park, L16 9JD, Liverpool, UK. Tel: (+44) 0151 2912184

Keywords: Antibiotic persistence, Recurrent infections, Folate, Antifolates, ampicillin, ofloxacin.

Subject category: Physiology and metabolism

Word count: 4709

Abbreviations: OCFM, folate one-carbon metabolism; 5-FCL, 5-formyltetrahydrofolate cyclo-ligase; TMP, trimethoprim; SMX, sulfamethoxazole; DMSO, dimethylsulfoxide; MIQE, Minimum information for publication of quantitative real-time PCR experiments; GCV, glycine cleavage complex;

Abstract

Bacteria can survive high doses of antibiotics through stochastic phenotypic diversification. We present initial evidence that folate metabolism could be involved with the formation of persisters. The aberrant expression of the folate enzyme gene *fau* seems to reduce the incidence of persisters to antibiotics. Folate impaired bacteria had a lower generation rate for persisters to both antibiotics ampicillin and ofloxacin. Persister bacteria were detectable from the outset of the exponential growth phase in the complex media. Gene expression analyses showed tentatively distinctive profiles in exponential growth at times when bacteria persisters were observed. Levels of persisters were assessed in bacteria with altered, genetically and pharmacologically, folate metabolism. This work shows that by disrupting folate biosynthesis and usage, bacterial tolerance to antibiotics seems to be diminished. Based on these findings there is a possibility that bacteriostatic antibiotics such as antifolates could have a role to play in clinical settings where the incidence of antibiotic persisters seem to drive recalcitrant infections.

INTRODUCTION

The capacity of microorganisms to survive or persist to antibiotics in a nonspecific manner, which is driven by phenotypic diversification rather than by genetic mutagenicity, has been shown to be of relevance in clinical settings [1, 2, 3, 4, 5]. Over the years evidence has gradually accumulated which suggests folates are involved in cellular growth regulation. For instance, folinic acid has been observed at high levels (≥70%) in dormant cellular forms, such as plant seeds and fungi spores [6, 7]. Moreover, the overexpression of 5-formyltetrahydrofolate cyclo-ligase (5-FCL), the enzyme that recycles folinic acid has been associated with bacterial dormant phenotypes [8, 9]. Intriguingly, inhibiting 5-FCL in eukaryotes affects cell growth [10, 11], and 5-FCL has been described as a pathogenic factor necessary for drug tolerance in *Mycobacterium* [12].

Folate metabolism can be nominally divided into three parts: *de novo* biosynthesis, the folate cycle (equated here to one-carbon folate metabolism or OCFM), and the intake of folate intermediates via facilitated membrane transport [13]. Folate products are involved in anabolic metabolism which is directly coupled with cellular replication activities, such as DNA biosynthesis, and is also associated with the production of methionine and NADPH [6, 13, 14]. The potential roles of folate metabolism in antibiotic persisters have significant pharmacological applications due to the well established role of antifolates within the pharmacopoeia of antimicrobial and anticancer treatment. Thus, exploring the metabolism of folates could offer venues to intervene and modify the phenotypic traits which facilitate microbial survival to antibiotics.

This study reports measurable levels of antibiotic persisters from the early exponential phase of bacterial growth. The *E. coli* folate gene knockout mutant which is lacking in 5-FCL (*Δfau*) presented lower levels of persisters in comparison to its reference isogenic strain BW25113. Next, we sought to determine if this effect could be observed by exposing fully functional strains to antifolate inhibitors. We found that both BW25113, as well as known hyper-persister *ΔhipA* had their survival to bactericidal antibiotics reduced. Finally, by adopting an optimised assay for the comparative detection of persisters, the gene expression for two different metabolic pathways (including a group of nutrient membrane transporters) was measured. Gene expression profiles had apparent differences among the metabolic states underlying those genetic and pharmacological alterations. These findings contribute to the development of a metabolic framework of the complex cellular states where persister phenotypes arise in actively growing cell populations [15].

METHODS

Bacterial strains

E. coli BW25113 (F-, *Δ(araD-araB)567, ΔlacZ4787* (::rrnB-3), *λ, rph-1, Δ(rhaD-rhaB)568, hsdR514*) is a direct derivative of K12 (BD792) used as the parent strain for the Keio Collection of single gene knockouts [16]. *E. coli* JW2879-1 is isogenic to BW25113 with the gene (*fau* or *ygfA*) encoding 5-FCL deleted (F-, *Δ(araD-araB)567, ΔlacZ4787* (::rrnB-3), *λ, ΔygfA763 ::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*). *E. coli* JW1500-2 is isogenic to BW25113 with the gene *hipA* deleted (F-, *Δ(araD-araB)567, ΔlacZ4787*(::rrnB-3), *λ, ΔhipA728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*). All three strains BW25113, JW2879-1 and JW1500-2 were procured from The Coli Genetic Stock Center. CGSC numbers 7636, 10233 and 9299, respectively.

Media and Culture

The liquid and solid complex media were Nutrient broth (70122, Sigma-Aldrich, UK) and Nutrient agar (70148, Sigma-Aldrich, UK), respectively. Strains were grown at 37ºC. Where stated M9 media was used as minimal media [17]. Liquid cultures were aerated by shaking in a water bath. Every assay reported here commencedwith different fresh cultures from frozen bacterial stocks. Cultures seeded with frozen stocks were incubated typically in 5 mL of culture for 6 to 8 hours under selective antibiotic pressure when appropriate (*Δfau* and *ΔhipA*). These cultures were then diluted 1000-fold in fresh media and incubated overnight. The following day cultures were diluted in fresh media at the same ratio again and used as fresh inocula left to grow up to the desired cell density (OD_{600}).

Antibiotics

Ofloxacin (Sigma O8757), ampicillin (Sigma A9518), and trimethoprim (TMP) (Sigma T7883) were dissolved in H_2O at stock concentrations of 50 mg/mL. Kanamycin (Sigma K1377) was dissolved in H_2O at 10 mg/mL, and sulfamethoxazole (SMX) (Sigma S7507) was dissolved in 10 % v/v dimethylsulfoxide (DMSO, Sigma 472301) at 50 mg/mL.

Growth curves

Fresh inocula were normalised to OD_{600} = 0.01 in fresh broth and cultured in 96-multiwell plates in 0.2 mL. The absorbance at OD_{600} was registered for 24 hours in a Thermo VarioSkan microplate reader (Thermo Fisher Scientific, MA, USA). For the three-hour growth assay overnight cultures were normalised to OD_{600} = 0.1 in 5 mL of fresh broth and aliquoted into as many samples as time points.

Antibiotic inhibitory concentrations

E. coli strains were pre-cultured as detailed in Media and Culture. Final cultures in either minimal or complex media were normalised to OD_{600} = 0.01 by triplicate in 96-well microplates. Antibiotics were present in four-fold dilutions. Growth controls were in triplicate samples without antibiotics. Background controls were included containing 5 mg/L of ofloxacin.

Three-hour persister assay

Fresh inocula were normalised at OD_{600} = 0.1 in 5 mL of fresh broth. Different samples were withdrawn from incubation at the following time points: 0, 0.25, 0.5, 1, 2 and 3 hours. Then either ampicillin at a final concentration of 100 mg/L or ofloxacin at 5 mg/L were added to each sample and incubated overnight (16-18 hours). All the different samples were then serially diluted 1:10 in 0.2 mL of 10 mM of $MgSO_4$ in 96-well microplates. Aliquots of 0.1 to 0.2 mL were plated out on media agar plates and incubated overnight for determination of colony-forming units (cfu) [18]. Where low number of persisters were expected (e.g., assays minimal media) the culture volumes were scaled up to obtain a minimal of approximately ten colonies per agar plate.

Three-hour antifolate assay

Fresh inocula were normalised to OD_{600} = 0.1 in 5 mL of fresh media. Either DMSO (0.1 % v/v), sulfamethoxazole (57.4 mg/L) or trimethoprim (1.7 mg/L) were added, final concentrations within parenthesis. These concentrations correspond to the reported steady-state mean levels of these two antifolates in human plasma during oral administration of Bactrim™ [19]. Samples were withdrawn from incubation at time points (in hours) 0, 0.25, 0.5, 1, 2 and 3. Aliquots of 1.5 mL from each samples were washed twice in fresh complex or minimal media. Samples were normalised to the lowest OD_{600} in 1 mL of media. Then ampicillin at final concentration of 100 mg/L or ofloxacin at 5 mg/L were added to each sample and incubated overnight (16-18 hours). All the different samples were then serially diluted 1:10 in 0.2 mL of fresh media in 96-well microplates. Aliquots of 0.1 mL were plated out on media agar plates and incubated overnight for determination of cfu.

Real-Time PCR gene expression analysis

Real-Time PCR (RT-PCR) workflow was carried out, taking into consideration the MIQE (Minimum information for publication of quantitative real-time PCR experiments) guidelines [20]. The *E. coli* BW25113 genome sequence [21] (GenBank accession number CP009273) was used to design PCR primers (Supplementary Table 16). Three different references genes were used: *cysG*, *hcaT* and *idnT* [22]. *E. coli* total RNA was extracted with PureZol (7326890, BioRad, UK) following the manufacturer's recommendations. The quality and quantity of RNA were assessed in the 2100 Bioanalyzer (Agilent Technologies, Santa Cruz, CA). Synthesis of cDNA was performed with iScript (Biorad, UK) in 0.02 mL reactions scaled up when required up to 0.12 mL. Amplification reactions were set up in 0.01 mL using SsoAdvanced Universal SYBR Green Supermix (Biorad, UK) following manufacturer's recommendations. PCR reactions were distributed in Hard Shell low profile skirted 96-well microplates sealed with Microseal B films (Biorad, UK). Amplification reactions (0.01 mL) were carried out in a CFX96 Real-Time System thermocycler (Biorad, UK), with the results compiled and automatically analysed at source with the CFX Manager software (Biorad, UK). The data presented represent three different biological replicates (n=3). Plated samples also included eight 6-fold dilutions of cDNA, with a known amount of DNA and using primers for *cysG*, for the generation of a standard curve. This allowed quantitation of gene amplification within each plate. Amplification efficiency was measured to assess the reproducibility of the amplification for each gene. Quantitative comparative gene amplifications was carried with normalised detection values and used to generate the fold changes as log ratios with change p-values (Supplementary Tables 17 - 19). The analysed data were compiled and plotted as heatmaps using heatmap2, which is a part of R's gplots [23].

Statistical analysis

Numerical analysis and graphical production were performed using R [23]. Antibiotic inhibitory concentrations were calculated with the dose-response analysis R extension package *drc* [24]. Principal Components Analysis (PCA) was conducted using the R packages FactoMineR and Factoextra [25]

RESULTS

Persistence to antibiotics is a phenotypic stochastic phenomenon that arises at low frequency in fresh media [26]. However, certain genetic backgrounds, such as the hyper-persister toxin *hipA* mutant [27, 28] or the altered gene expression of certain genes [8] have been shown to influence the frequency of persisters. An example of the latter is the decrease in the rate of persisters under low expression of the gene that encodes for the folate enzyme 5-FCL [8]. The *E. coli* gene knockout strains *Δfau* and *ΔhipA* and their isogenic parental strain (BW25113) were used throughout this work. Thus, we first measured the sensitivity of these three strains to each of the different antibiotics used in this study in both complex and minimal media. Expectedly, there were high inhibitory concentrations (IC_{50}) of SMX in the complex media and the folate knockout strain *Δfau* was comparatively more sensitive to SMX in the minimal media (Table 1, Supplementary Figs. 1 and 2).

Importantly, the concentrations of ampicillin and ofloxacin used for the selection of persisters were above the IC_{50} observed for these strains. Also, the concentrations of antifolates (with the exception of SMX in complex media) used here were above the inhibitory concentrations (Table 1). Significantly, the inhibitory concentrations of antifolates in vitro were not decisive for the concentrations of antifolate to use in the persister assays. We sought to measure the effect of antifolates at concentrations in line with to the steady-state levels found in human plasma during treatment with oral formulation of SMX and TMP [19].

Growth rates

We were primarily interested in studying persisters arisen under non apparent stress (i.e., cells in logarithmic growth in fresh media), also known as Type II persisters [3], given the known phenotypic diversification in growth rate [29] and cell mass [30] in the logarithmic phase which we hypothesise can be relevant to persister formation. However, and in contrast to previous investigations [8, 27, 28], it was found that the growth rate in *Δfau* and *ΔhipA* was different to the parental strain, BW25113, in a time and media-dependent fashion (Fig. 1). It was deemed necessary to establish incubation times that allowed observations to be made without visible differences in growth rate. Batch cultures were followed throughout 3 hours with a starting OD_{600} = 0.1, optimised to allow the retrieval of cells growing logarithmically in either complex or minimal media (Fig. 1). Noticeably, the generation time approximately doubled in the minimal media (1.5 ± 0.13 hours), in comparison to bacteria which grew in the complex media (0.66 ± 0.07 hours) for all three strains (Fig. 1). Importantly, within this time window and at the observed cell densities no differences in growth rates among strains were observed, independent of the type of media and the cell counts per unit of OD_{600} were comparable among strains, BW25113 (95% CI 4.9 - 5.1 x 10^8 cells), *Δfau* (95% CI 4.9 - 5.2 x 10^8 cells) and *ΔhipA* (95% CI 5.0 - 5.15 x 10^8 cells).

Biphasic response to ampicillin and ofloxacin

Low-level-persistence phenotypes are usually observed as biphasic responses to antibiotics. At a OD_{600} = 0.3, both the reference and the folate mutant strains displayed a time-dependent killing level of persisters which stabilised after 6 hours of incubation in ampicillin in either complex or minimal media (Fig. 2). The folate mutant continued to show a decrease of persisters to ampicillin in complex media after 6 hours. At 16 hours we measured the incidence of persisters to ampicillin at 95% CI 320 - 400 cfu per 10^8 cells for BW25113 and at 95% CI 56 - 80 per 10^8 cells for *Δfau*. A similar pattern, but with fewer persisters, was seen for ofloxacin in BW25113 (95% CI 8 - 20 per 10^8 cells) and *Δfau* (95% CI 4 - 8 per 10^8 cells). In minimal media levels of persisters were equivalent to single digits per millilitre (Fig. 2, Supplementary Table 2). Thus, follow-up experiments in minimal media were carried scaling up culture volumes to obtain the equivalent to a minimum of ten colonies per agar plate to calculate cfu per millilitre (cfu/mL).

Rate of persistence in cells in logarithmic growth (the 3-hour assay)

The appearance rate of persisters is presented as ratios of cfu/mL from a given batch culture at each time over the cfu/mL of the batch culture at time zero. This normalises the persister phenotypes related to the physiological status of the inoculates at the starting point. By three hours in complex media BW25113 had twice more persisters to ampicillin than *Δfau* (Fig. 3a). BW25113 presented again more persisters to ofloxacin than *Δfau*, this time by near a five-fold difference (Fig. 3b). Remarkably, the number of persisters for either strain increased consistently from their lag phase and along their growth in exponential phase. An observation in line with the reported active generation of persisters to antibiotics in rapidly growing *E. coli* [15]. In sharp contrast, the ratio of persisters in the minimal media for BW25113 and *Δfau* declined throughout, and were closed to zero by 3 hours. This applied to both, bacteria exposed to ampicillin (Fig. 3c) and ofloxacin (Fig. 3d). The metabolic network of cells in minimal media was clearly incompatible with the necessary metabolic state required for the phenotypic diversification that drives persistence.

The effect of antifolates on the reference strain BW25113

It was considered relevant to study how the results presented thus far compared with the behaviour of the bacteria following administration of pharmacological inhibitors of folate biosynthesis, and how this affected the development of antibiotic persisters. In the absence of validated inhibitors of 5-FCL it was deemed cogent to use two well established antimicrobial antifolates: sulfamethoxazole (SMX) and trimethoprim (TMP). Inhibitors of the *de novo* folate biosynthesis enzyme dihydropteorate synthetase, and the OCFM enzyme dihydrofolate reductase, respectively. The BW25113 strain was incubated for up to three hours using each of these antifolates or DMSO as the control for the solvent used for SMX. As expected, growth was affected by TMP in the complex media and by both SMX and TMP in the minimal media (Figs. 4a and 4d). Therefore, samples had to be normalised based on their optical densities to the sample with the least cell mass per volume. Thereafter, cultures were exposed overnight to either ampicillin or ofloxacin.

Incubation with TMP in complex media repressed the development of persisters to both ampicillin and ofloxacin (Figs. 4b and 4c). The effect of SMX is not apparent for ampicillin but interestingly it appear to have reduced persisters to ofloxacin after two hours (Fig. 4c). Bacteria in minimal media was still affected by the incubation in SMX given the lower ratio of persister for ampicillin than in the solvent control and TMP (Fig. 4e). The ratios of persisters for ofloxacin were reduced by TMP and less so by SMX in comparison to DMSO (Fig. 4f). On the other hand, it seems that 0.1 % DMSO was sufficient to cause a noticeable drop, by approximately an order of magnitude, in the ratio of persisters by three hours (Figs. 4b and 4c) in comparison to the levels observed before in complex media (Figs. 3a and 3b). Nonetheless, and particularly for TMP in complex media, the number of persisters quantified by three hours of incubation in this antifolate allowed us to detect 10-fold and 50-fold less persisters than in the control for ampicillin and ofloxacin, respectively (Figs. 4b and 4c). In minimal media TMP showed a 10-fold difference in comparison to the control for ofloxacin (Fig. 4f). Thus, in complex media TMP seems to reduce the number of persisters to ampicillin and ofloxacin while in minimal media TMP causes mainly a visible, though lesser, reduction in ofloxacin persisters.

The effect of antifolates on the hyper-persistent mutant strain *ΔhipA*

By the very nature of the stochasticity of phenotypic diversification, microbes are likely to be in a broad spectrum of physiological states while growing logarithmically. It was reasoned important to investigate the response to antifolates from a strain prone to develop persisters by using the knockout mutant of toxin hipA (*ΔhipA*), a well known hyper-persister [27, 28]. In contrast to SMX, TMP had a discernible inhibition on the growth of *ΔhipA* (Fig. 5a). Subsequently, TMP showed to have a clear suppressing effect on the ratio of persisters in *ΔhipA* in complex media for both ampicillin (approximately 50-fold reduction) and ofloxacin (approximately 500-fold reduction) (Figs. 5b and 5c), in a trend not dissimilar to the effects on the parental strain (Figs. 4b and 4c). In minimal media both SMX and TMP reduced the growth rate of *ΔhipA* (Fig. 5d). Both antifolates also reduced the ratios of persisters in this media for ampicillin and ofloxacin (Figs. 5e and 5f), with a more pronounced reduction in persister levels for ofloxacin (Fig. 5f).

The impact of Antifolates on persister development: Principal Components Analysis

Principal Components Analysis (PCA) was used to summarise the data gathered for the effects of antifolates BW25113 and *ΔhipA*. Data were organised as listed in the Supplementary Table 15: presence of antifolates, type of antibiotic (ampicillin or ofloxacin), strain (BW25113 or *ΔhipA*), time spent in antifolates (0 - 3 hours), media (complex or minimal), and ratio of persisters (cfu/mL at a given time up to 3 hours over cfu/mL at time zero). The first two dimensions made up approximately 41% of the total variance of the dataset (inertia) (Fig. 6).

The variables type of antibiotic and the time spent in antifolates were correlated positively to the ratio of persisters. This was a key finding, as it suggested persister development was associated with the type of antibiotic (ampicillin or ofloxacin) in agreement with the literature, but also here in relation to the time spent in the antifolates. The variables media and the type of antifolates were negatively correlated to the ratio of persisters, indicating that in either media either antifolate seemed to lower the incidence of persisters (Fig. 6). Notably, the variable strain, the reference strain BW25113 or the hyper-persister *ΔhipA*, behaved as a supplementary (*i.e.*, non-active) variable. This suggested antifolates had a similar effect on both of these strains. That is to say the hyper-persister strain as susceptible as the reference strain to the effects of antifolates. An encouraging finding for clinically relevant settings.

The gene expression profile of the reference strain BW25113 in exponential growth

It was logical to investigate the molecular basis for the development of persisters in the reference *E. coli* strain. A methodology was developed to use optimised targeted RT-PCR amplifications to profile the gene expression responses of the parental *E. coli* strain, BW25113 throughout the 3-hour growth assay in the complex media (Fig. 1c). Data were analysed and interpreted by comparing the gene expression levels at different time points against the initial samples (time zero). Cells that grew within this time frame displayed the persistence phenotypes associated with both ampicillin and ofloxacin (Figs. 3a and 3b). The set of genes which were queried encode for folate biosynthesis and usage (OCFM) pathways, fifteen solute transporters, twelve fatty acids biosynthesis enzymes, and the *cydX* gene (subunit X of cytochrome d (bd-I) ubiquinol oxidase) (Supplementary Table 16). Also included were fatty acid biosynthesis genes which have been shown to be involved in the development of persisters [31].

On the other hand, oxidative phosphorylation, represented here by *cydX*, has been shown to sensitise bacteria to antibiotics [32]. Consistently, cydX was underrepresented in exponential growth phase, along the generation of persisters (Figs. 3a, 3b and 7). This is in agreement with glycolysis being the main source of cell mass and energy in rapid cell growth with the flux control for this pathway originating mainly from ATP utilisation due to the fact that the majority of the control of growth rate resides in the anabolic reactions [33].

Folate biosynthesis represented by *folC, pabC, aroH* and *folP* appeared to be active through the initial hour of growth in fresh complex media (Fig. 7). Only the gene expression of the transporters of amino acids and pantothenate, *putP* and *panF*, seem to have increased before the initial hour. After the first hour, the expression of genes representing other enzymes of folate biosynthesis as well as nine more substrate membrane transporters seemed to have increased (*cycA, shiA, citT, nupC, dsdX, nupG, aroP, glcA*, and *uhpT*) (Fig. 7).

The comparative gene expression profile of the folate mutant strain *Δfau* in exponential growth

We assessed the genetic expression profile of the gene knockout folate mutant *Δfau* in comparison to BW25113. The initial outline at time zero showed the majority of genes underexpressed with three genes overexpressed in *Δfau* cells, all three solute membrane transporters (*gadC, uhpT, citT*) involved in the uptake of organic acids and carbohydrates (Fig. 8). During the first hour there was an apparent overexpression of genes that included the glycine cleavage complex (GCV) of the OCFM (*lpdA, gcvH, gcvP, gcvT*), whose gene expression pattern clustered with the proline:Na symporter *putP*. The time points at two and three hours (Fig. 8) gave an indication of the potential overexpression of a number of genes involved in encoding lipogenesis enzymes. The majority of solute membrane transporters targeted here were overexpressed by the second or third hour (*shiA, nupG, gadC, nupC, cycA, panF, uhpT, glcA, aroP, citT, adeQ,* and *dsdX*). Altogether, folate metabolism in the folate mutants was underrepresented, while lipogenesis, and a number of solute membrane transporters seem to have been overrepresented.

The comparative gene expression profile of the bacteria exposed to trimethoprim

In the complex media only TMP was found to alter the incidence of persisters to ampicillin and more significantly to ofloxacin (Fig. 4). BW25113 cells treated with TMP had initially a discrete number of overrepresented genes encoding folate metabolism and lipogenesis together with two membrane transporters (*aroP* and *adeQ*) (Fig. 9). After one hour, the over-expression of genes encoding for folate metabolism (particularly OCFM) and lipogenesis became more apparent (Fig. 9). Similarly, five solute membrane transporters were expressed after the first hour (*cycA, pnuC, panF, adeQ*, and *nupG*). Contrary to the *Δfau* gene knockout strain, the response to antifolate treatment seems to have included a representation of folate biosynthesis (Figs. 8 - 9).

DISCUSSION

The evidence presented here connects folate metabolism to the generation of persisters to antibiotics in exponentially growing bacteria. Antibiotic persisters were described soon after the discovery of penicillin [34] and subsequently observed across different forms of drug tolerance in human pathogens [3, 4, 35]. However, it is unknown how sub-populations of microorganisms survive environmental stressors and qualify as persisters. In an attempt to conceptualise this phenomenon some have called persistence a consequence of dormancy or latency. Here, we are avoiding these terms which may not necessarily convey the physiology of cells in rapid growth that happen to survive a given stressor [15, 36]. In cells under low nutrient levels (i.e., bacteria in spent media), the development of persisters to antibiotics is three to four orders of magnitude higher than in a population in fresh media [37, 38]. This has been rationalised under the known cell responses that takes place under scarcity of nutrients [39]. However, bacteria viable after exposure to antibiotics are also observed in steady-state growth (Type II persisters) before shortage of nutrients becomes significant (e.g., stationary phase in batch culture) [15, 40, 41]. Here, we have observed that *E. coli* with either genetically or pharmacologically impaired folate metabolism, presented low levels of persisters. This is a counterintuitive finding since interrupting anabolic metabolism and slow cell growth (i.e., exposure to bacteriostatics such as antifolates) is expected to increase the level of persisters [1 - 3].

We observed that persisters to antibiotics are detectable from the moment bacteria leave the lag phase in complex media. A trend that continues along the logarithmic growth (Fig. 3). This is an observation in agreement with previous findings [15]. We propose that the source of the phenotypic variability, a requirement for the appearance of persisters, could be explored under the known cell to cell heterogeneity intrinsic to rapid cell growth. Namely, cell to cell and within cell gene expression variability [42], variability in DNA and protein contents [43], as well as cell size variability [30]. On the other hand, the functions of folate cofactors involve providing carbon units to anabolic pathways for DNA biosynthesis, methionine biosynthesis, and NADPH production [14, 44]. Consequently, antifolates such as TMP produce cellular stasis and death by reducing cellular levels of the pyrimidine thymidine which desarranges DNA replication and triggers a futile cycle of DNA damage and repair ("thymineless death") [45]. At this point the basis of our findings become more apparent since interfering with folate metabolism reduces the concentration of metabolites required for such key anabolic processes directly linked to cell growth. Consistent with this, the gene encoding carbamoyl phosphate synthetase (involved in the *de novo* biosynthesis of pyrimidines) has been singled out in a recent genetic scan where its loss of function mutant reduced thousands-fold the generation of persisters to antibiotics in bacteria [46].

Supporting the above, batch cultures of the reference strain in minimal media at cell densities equivalent to that in complex media showed significantly less persisters to either ampicillin or ofloxacin by approximately two orders of magnitude (Figs. 2 and 3). We interpret this as a consequence of reduced metabolite availability which has an impact on the rate of anabolism necessary for cell growth and multiplication (e.g., DNA replication, transcription, protein biosynthesis, ribosomal mass). For instance, disarrayed replication forks under scarcity of pyrimidines and the deleterious effects of uracil incorporation into DNA from thymidine-deficient nucleotide pools induces a low frequency of initiation of the replication cycle and origin-proximal DNA degradation during thymine starvation [45]. The reduction of metabolites that impact cell growth limits the phenotype landscape from which persisters arise. Under abundant nutrients the stochastic differences among individuals in, for instance, rate of processes such as DNA initiation and elongation, would provide a broad phenotypic diversity. Contrarily, under limited sources a low metabolic flux would only sustain low anabolic rates that attenuates intracellular and intercellular stochastic effects.

In complex media specific nutrient transport into the cell becomes advantageous and membrane transport has been shown to be part of the development of persisters [47, 48]. The results of this investigation seem to suggest that certain substrate membrane intake occurs along logarithmically growing bacteria (Figs. 7 - 9). Several studies have used experimental antimetabolites to ameliorate the appearance of bacterial persisters [49, 50, 51, 52, 53, 54]. A great advantage of antimetabolites such as antifolates is the fact that they are licensed antimicrobials in clinical practice and have been so for eight decades. Although new antifolates have been added to the cancer chemotherapy pipeline [55], the development and deployment of new antifolates as antimicrobials lags significantly behind [56]. Nonetheless, evidence continues to support the repurposing of antifolates in the treatment of infectious diseases, including those where resistance has become widespread [57, 58, 59]. It is encouraging to have found that antifolates affected a known hyper-persister *E. coli* strain as much as the reference strain. This is of relevance for the clinical scenarios where it is likely to find a range of persister levels in active infections. If the appearance of phenotypic variants that persist to antibiotics is closely coupled with the dynamics of exponential generation of cellular mass in *E. coli*, folate metabolism would have a role in the cellular metabolism underlying such variability. Therefore, antifolates could offer a potential avenue for assisting in improving the success of current bactericidal antibiotic treatments.

Funding information

HEFCE research funding, Liverpool Hope University, UK.

Acknowledgements

The project was possible thanks to the support of Liverpool Hope University, School of Health Sciences. Quality control of RNA was implemented with the collaboration of Dr Clare Strode at Edge Hill University, UK.

Conflict of interests

The authors declare that they have no competing interests.

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Figure legends

Fig. 1. Growth rates. Bacterial growth was followed for 24 hours in either complex media (a) or minimal media (b). BW25113 denotes the reference strain, and *Δfau* and *ΔhipA* the gene knockout strains for the folate gene *fau* and the toxin gene *hipA*, respectively. Bacterial growth was also followed for 3 hours in either complex media (c) or minimal media (d). The Y-axes represent the optical density (OD_{600}) with a starting OD_{600} = 0.1. The means and standard deviations for three different experiments (n = 3) are shown.

Fig. 2. Biphasic time-dependent killing. Cultures were grown to an OD_{600} = 0.3 before adding either ampicillin (100 mg/L) or ofloxacin (5 mg/L) in complex media (a) or minimal media (b) for the indicated times. Samples collected at a given time were diluted and spread plated on LB agar. The experiments were performed in triplicate (n=3). Data compiled in the Supplementary Tables 1 and 2.

Fig. 3. The incidence of persisters in the folate gene knockout strain in complex media. Bacteria were exposed for 16 hours to ampicillin 100 mg/L (a) or ofloxacin 5 mg/L (b), after growing in complex media for different durations (0, 0.25, 0.5, 1, 2 and 3 hours). Bacteria in minimal media were also exposed to ampicillin 100 mg/L (c) or ofloxacin 5 mg/L (d). The left Y-axes show the optical density of the cultures and the right Y-axes show the ratios (versus time zero) of persisters after two hours of incubation in either antibiotic. Data compiled in the Supplementary Tables 3 - 6.

Fig. 4. The effects of antifolates on persistence to antibiotics in the reference strain BW25113. (a) Bacteria in complex media in the presence of either 0.1% (v/v) dimethylsulfoxide (DMSO), 1.7 mg/L trimethoprim (TMP), or 57.4 mg/L sulfamethoxazole (SMX). At different time points (0, 1, 2, and 3 hours) the cultures were washed, resuspended in complex media and incubated for 16 hours in the presence of either antibiotic, ampicillin 100 mg/L (b) or ofloxacin 5 mg/L (c). Incidence of persister is shown as a ratio of cfu/mL from each time point over time zero. (d) Bacteria in minimal media in the presence of either 0.1% (v/v) dimethylsulfoxide (DMSO), 1.7 mg/L trimethoprim (TMP), or 57.4 mg/L sulfamethoxazole (SMX). At different time points (0, 1, 2, and 3 hours) the cultures were washed, resuspended in minimal media and incubated for 16 hours in the presence of ampicillin 100 mg/L (e) or ofloxacin 5 mg/L (f). Incidence of persister is shown as a ratio of cfu/mL from each time point over time zero. Data compiled in Supplementary Tables 7 - 10.

Fig. 5. The effects of antifolates on the persistence to antibiotics in the hyper-persister strain *ΔhipA*. (a) Bacteria in complex media in the presence of either 0.1% (v/v) dimethylsulfoxide (DMSO), 1.7 mg/L trimethoprim (TMP), or 57.4 mg/L sulfamethoxazole (SMX). At different time points (0, 1, 2, and 3 hours) the cultures were washed, resuspended in complex media and incubated for 16 hours in the presence of either antibiotic, ampicillin 100 mg/L (b) or ofloxacin 5 mg/L (c). The incidence of persisters is shown as a ratio of cfu/mL from each time point over time zero. (d) Bacteria in minimal media in the presence of either 0.1% (v/v) dimethylsulfoxide (DMSO), 1.7 mg/L trimethoprim (TMP), or 57.4 mg/L sulfamethoxazole (SMX). At different time points (0, 1, 2, and 3 hours) the cultures were washed, resuspended in minimal media and incubated for 16 hours in the presence of ampicillin 100 mg/L (e) or ofloxacin 5 mg/L (f). The incidence of persisters is shown as a ratio of cfu/mL from each time point over time zero. Data compiled in the Supplementary Tables 11 - 14.

Fig. 6. Principal Components Analysis for the effect of antifolates on the development of persisters. The dataset on antifolates was summarised with six different variables: strain (BW25113 or *ΔhipA*), time exposure to antifolates (in hours), antifolate (DMSO, SMX, TMP), media (minimal media or complex media), antibiotic (ampicillin or ofloxacin), and level of persisters (the ratio of cfu/mL at each time point in antifolates over the cfu/mL at time zero). The X-axis represents the principal component 1, and the Y-axis represents the principal component 2. Distances from zero represent the magnitudes of the effects of each variable. The associated ranks in order to carried out the PCA are in Supplementary Table 15.

Fig. 7. Gene expression in the reference strain (BW25113) during the three-hour growth assay in complex media. The levels of gene expression, at the specified time points (in hours), as a ratio of the values at time zero. The yellow column at the initial time point denotes no differences in gene expression since this the ratio of gene expression levels in this sample against itself. Colour keys for the metabolic pathways which were studied are to the left of the heatmap (respiration gene *cydX* denoted by the black colour key) and the gene list is to the right.

Fig. 8. Comparative differential gene expression in the folate mutant strain. The levels of gene expression, at the specified time points (in hours), in the folate knockout mutant *Δfau* strain compared to the reference strain BW25113. Colour keys for the metabolic pathways which were studied are to the left of the heatmap (respiration gene *cydX* denoted by the black colour key) and the gene list is to the right.

Fig. 9. Gene expression in the reference strain (BW25113) in the presence of trimethoprim. Gene expression, at the specified time points (in hours), in the BW25113 strain incubated in trimethoprim (1.7 mg/L) when compared to the untreated BW25113 control. The yellow column at the initial time point denotes no differences in gene expression at the moment of adding the antifolate. Colour keys for the metabolic pathways which were studied are to the left of the heatmap (respiration gene *cydX* denoted by the black colour key) and the gene list is to the right.

Tables

Table 1. The inhibitory concentrations of antibiotics and the solvent (DMSO) used in this study. All three strains of *E. coli* are included. BW25113 is the parental strain, *Δfau* is the folate mutant strain, and *ΔhipA* is the hyper-persister strain. Data represent inhibitory concentrations as IC_{50} in mg/L, except for DMSO which is in % (vol/vol).

	Minimal media			**Complex media**			
	BW25113	***Δfau***	***ΔhipA***	**BW25113**	***Δfau***	***ΔhipA***	**Used**
DMSO	5.7 ± 0.67	13.7 ± 0.47	6.62 ± 0.41	8.173 ± 1.743	5.746 ± 0.952	7.166 ± 1.98	0.1
Ampicillin	3.96 ± 0.3	3.93 ± 0.423	2.31 ± 0.017	6.61 ± 0.718	5.644 ± 0.563	4.969 ± 0.499	100
Ofloxacin	0.234 ± 0.004	0.234 ± 0.0043	0.249 ± 0.003	0.084 ± 0.023	0.095 ± 0.015	0.088 ± 0.007	5
Trimethoprim	0.59 ± 0.027	0.539 ± 0.022	0.62 ± 0.011	0.572 ± 0.082	1.064 ± 0.242	0.899 ± 0.138	1.7
Sulfamethoxazole	0.39 ± 0.023	0.132 ± 0.01	0.464 ± 0.032	349 ± 8.55	141 ± 2.83	2383 ± 262	57.4

Additional Files

Additional file 1 — Inhibitory concentration curves in minimal media of antibiotics and DMSO on the *E. coli* strains used in this study.
Additional file 2 — Inhibitory concentration curves in complex media of antibiotics and DMSO on the *E. coli* strains used in this study.
Additional file 3 — Supplementary tables 1 to 16
Additional file 4 — Supplementary table 17 gene expression BW25113
Additional file 5 — Supplementary table 18 gene expression fau versus BW25113
Additional file 6 — Supplementary table 19 gene expression TMP-treated BW25113 versus BW25113

Figure 1

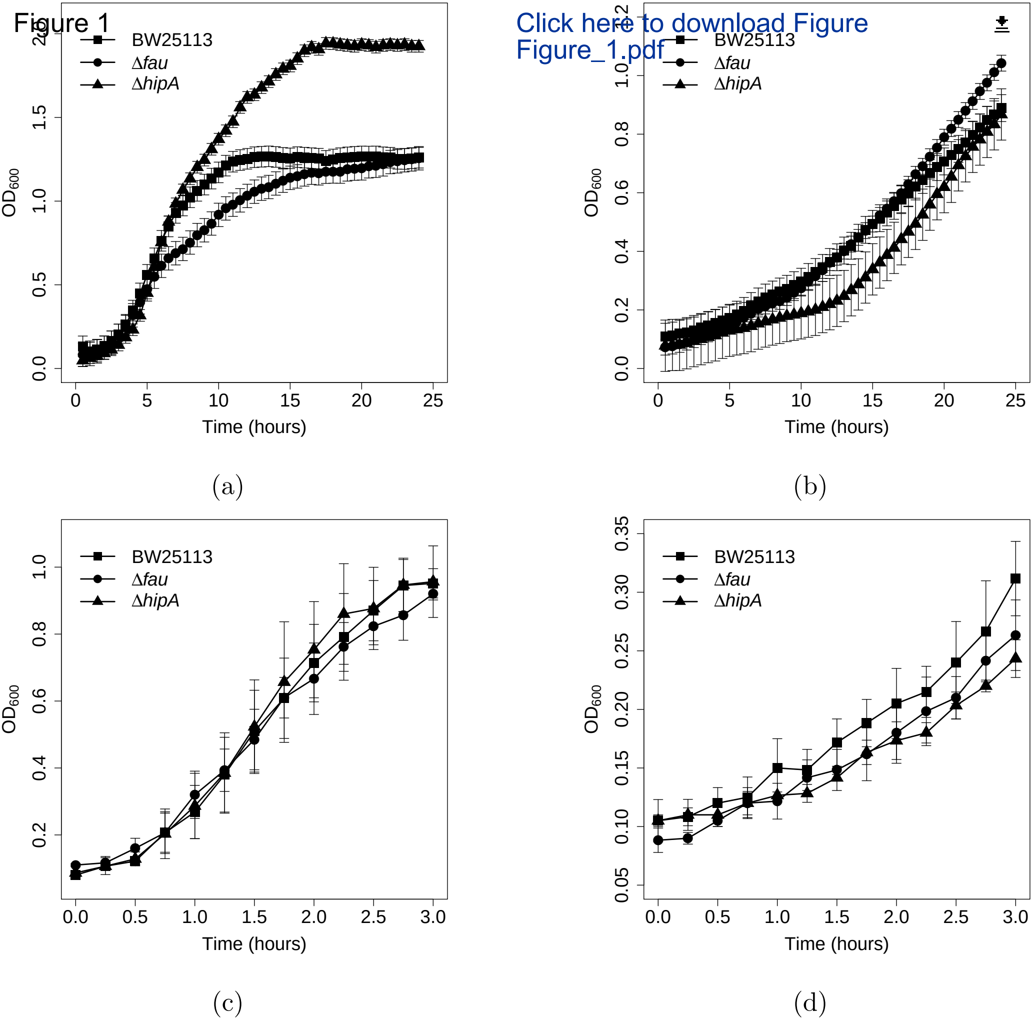

Figure 2

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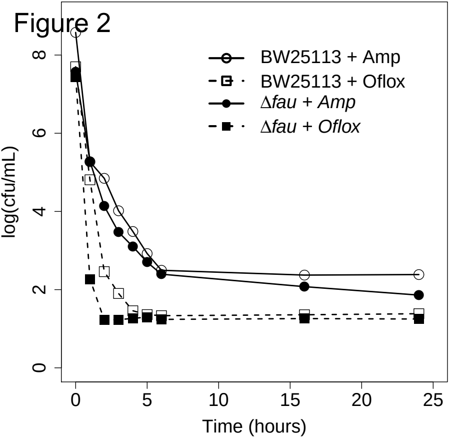
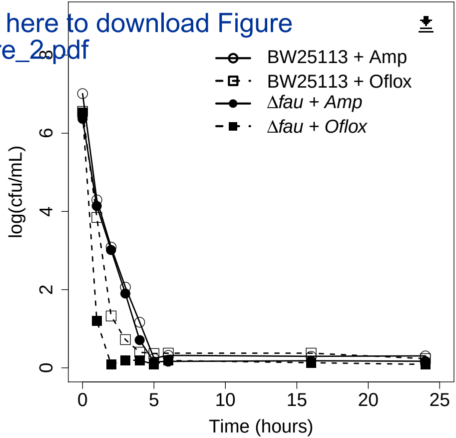
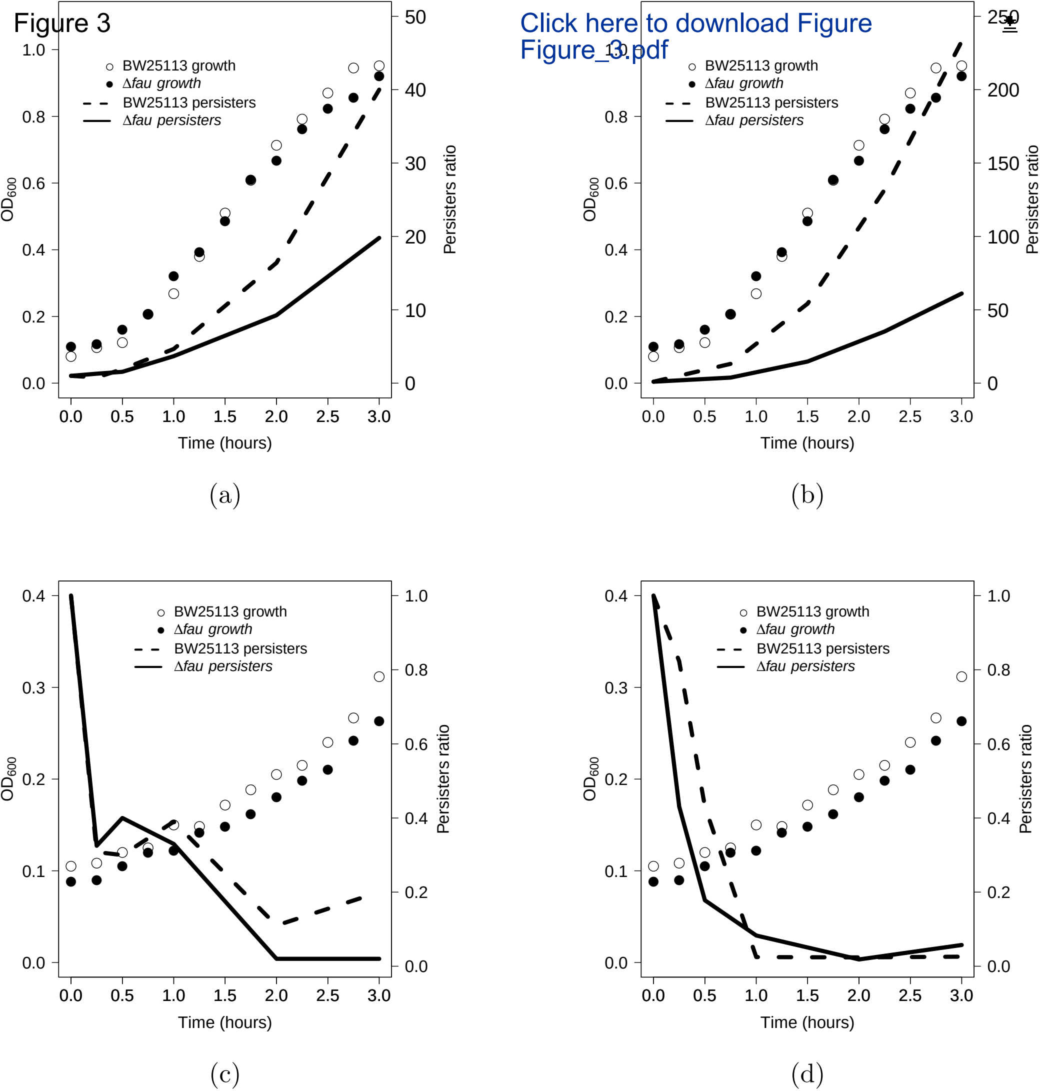


(a)



(b)

Figure 3



BW25113 growth
Δfau growth
BW25113 persisters
Δfau persisters
OD600
Persisters ratio
Time (hours)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50
50
100
150
200
250
0.1
0.3
(a)
(b)
(c)
(d)

Figure 4

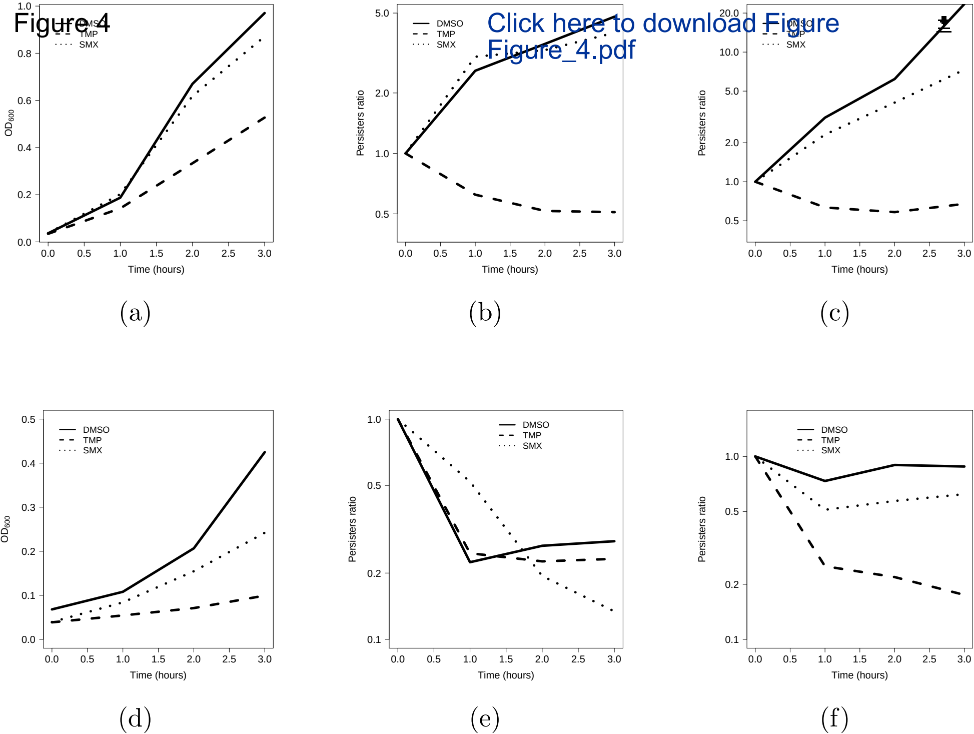

DMSO
TMP
SMX
OD600
Time (hours)
(a)
Persisters ratio
Time (hours)
(b)
Persisters ratio
Time (hours)
(c)
OD600
Time (hours)
(d)
Persisters ratio
Time (hours)
(e)
Persisters ratio
Time (hours)
(f)

Figure 5

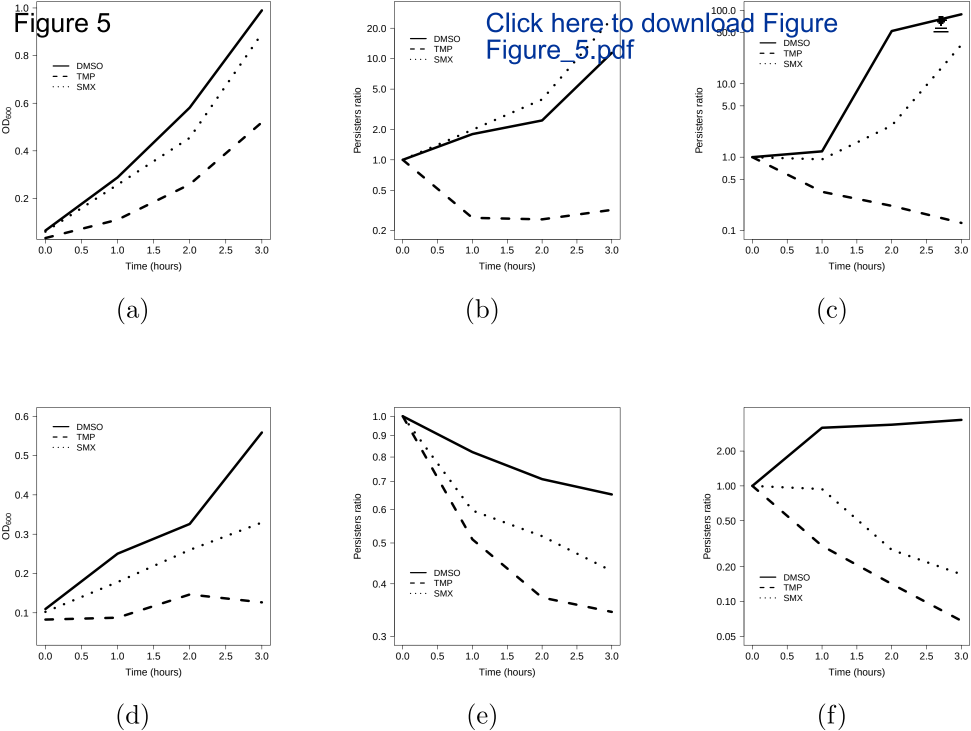


Figure 6

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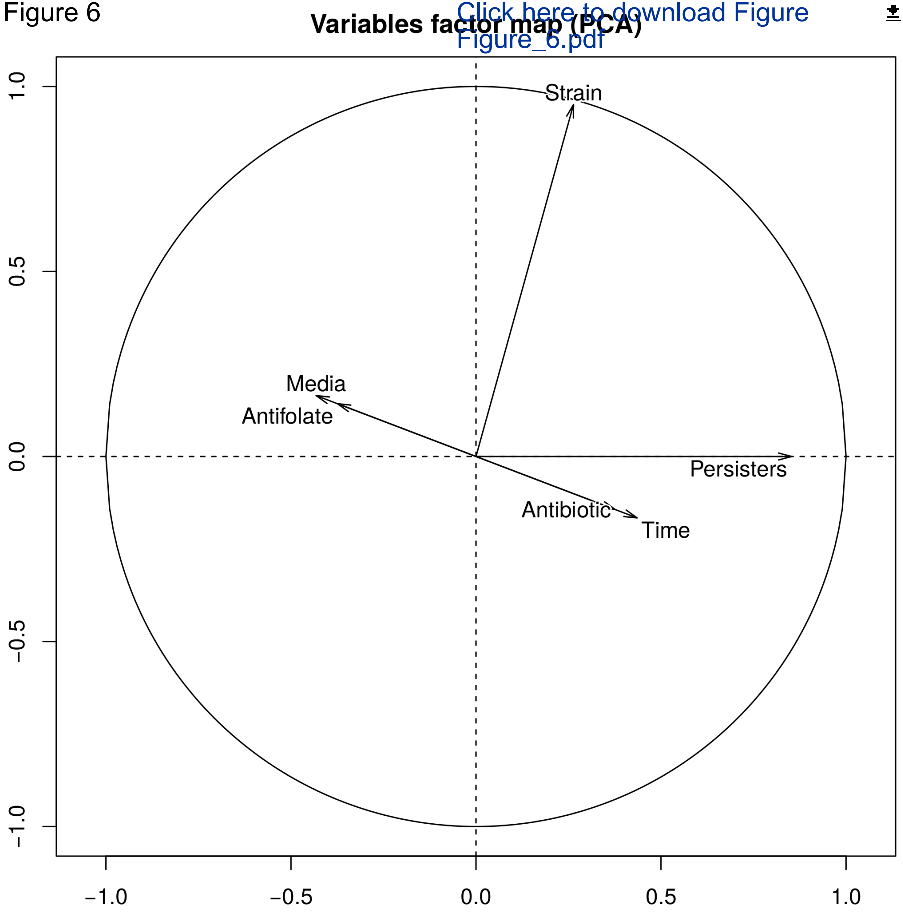

Figure 7

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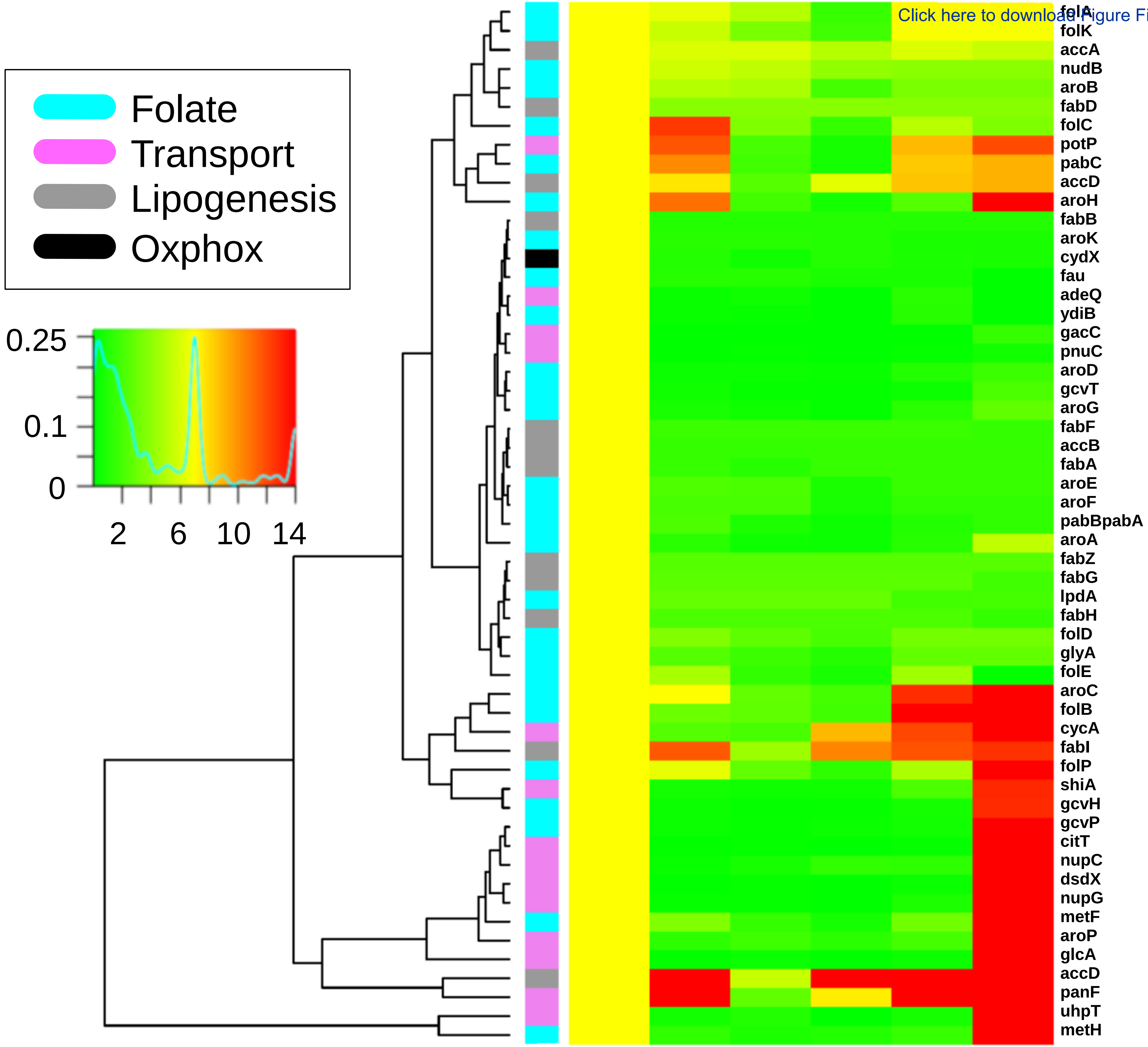
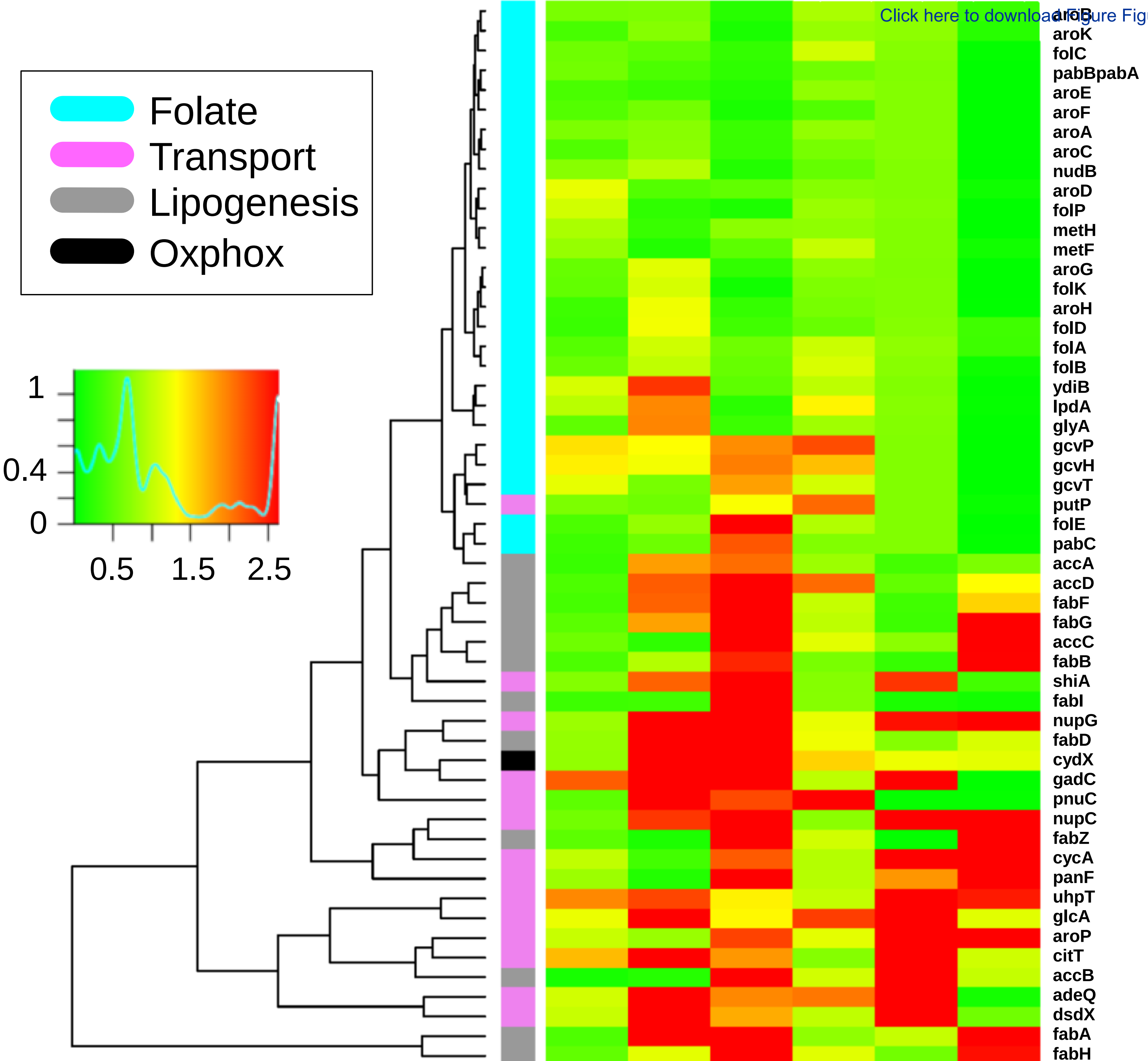


Figure 8



Folate
Transport
Lipogenesis
Oxphox
1
0.4
0
0.5
1.5
2.5
aroK
folC
pabBpabA
aroE
aroF
aroA
aroC
nudB
aroD
folP
metH
metF
aroG
folK
aroH
folD
folA
folB
ydiB
lpdA
glyA
gcvP
gcvH
gcvT
putP
folE
pabC
accA
accD
fabF
fabG
accC
fabB
shiA
fabI
nupG
fabD
cydX
gadC
pnuC
nupC
fabZ
cycA
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Figure 9

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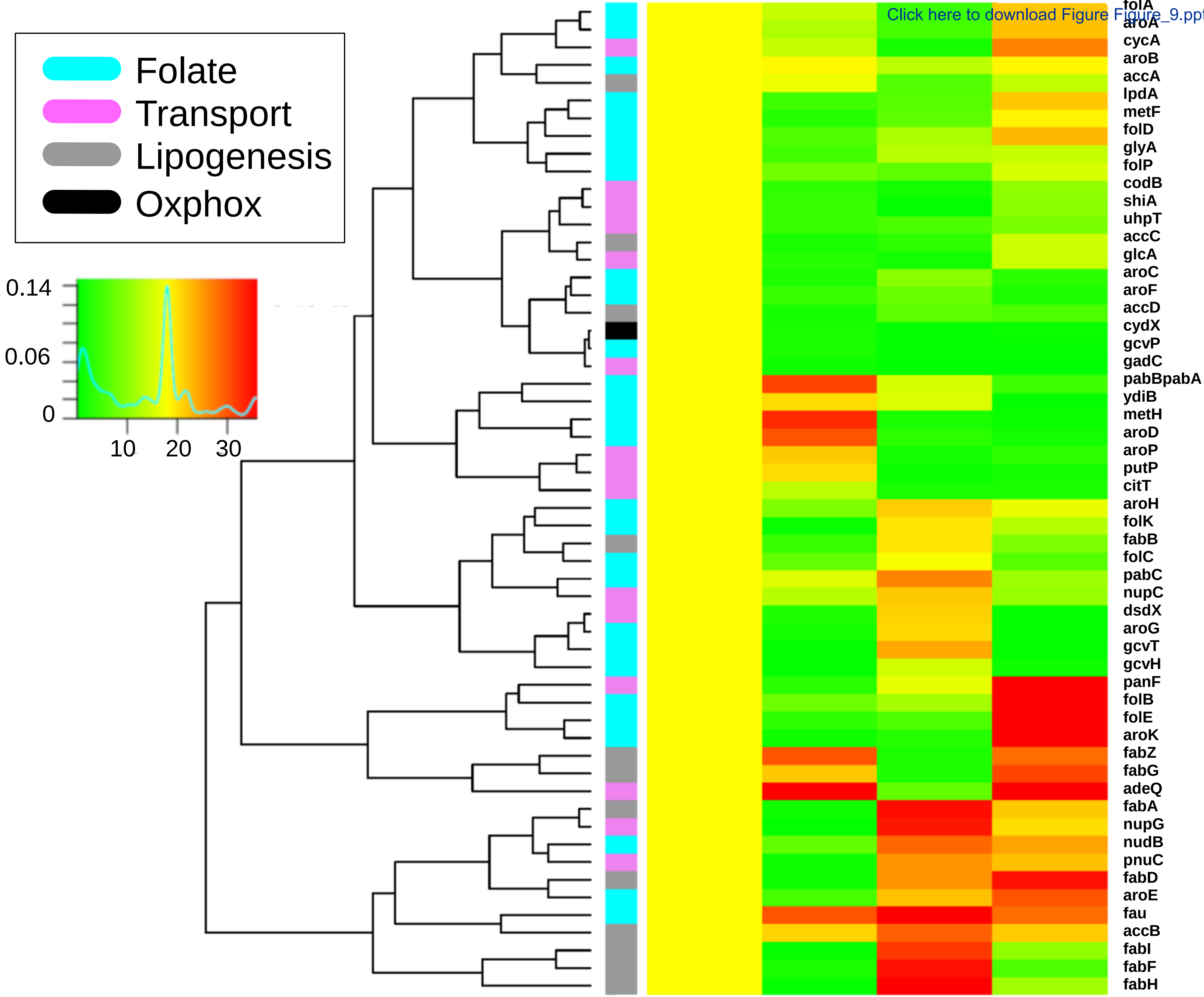
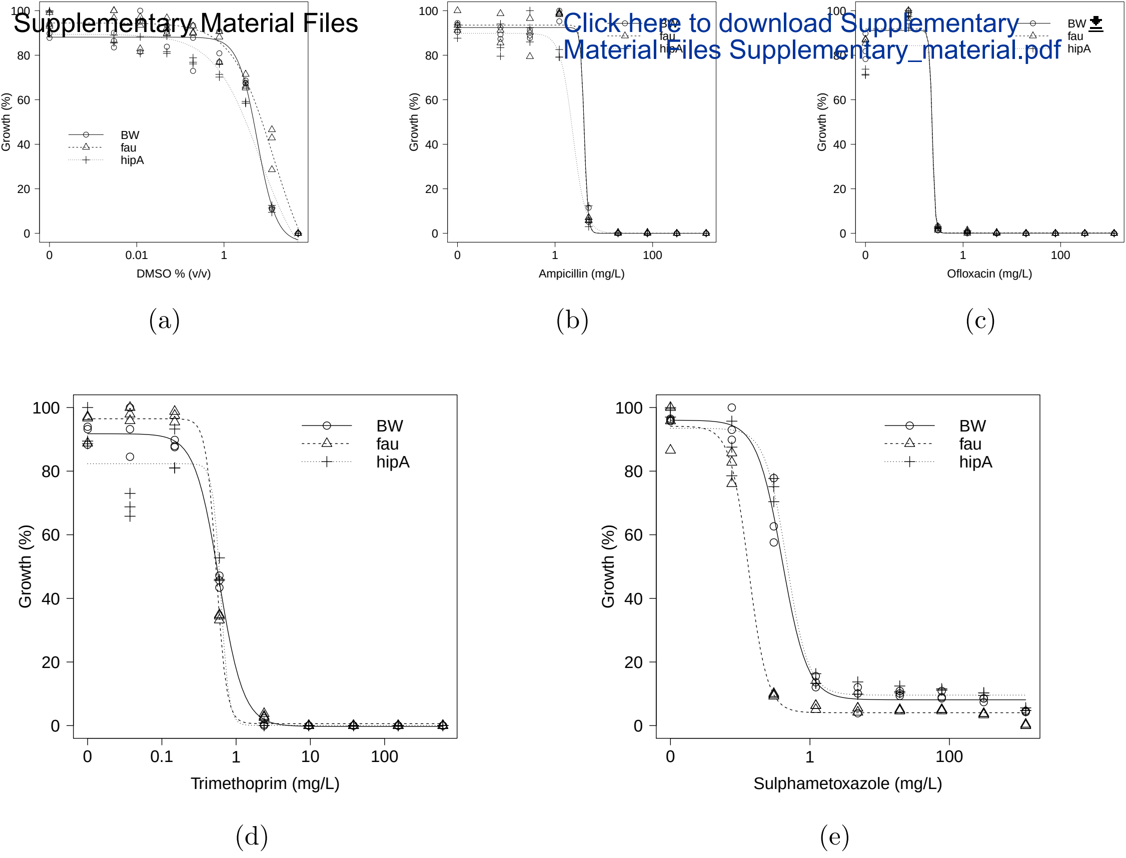
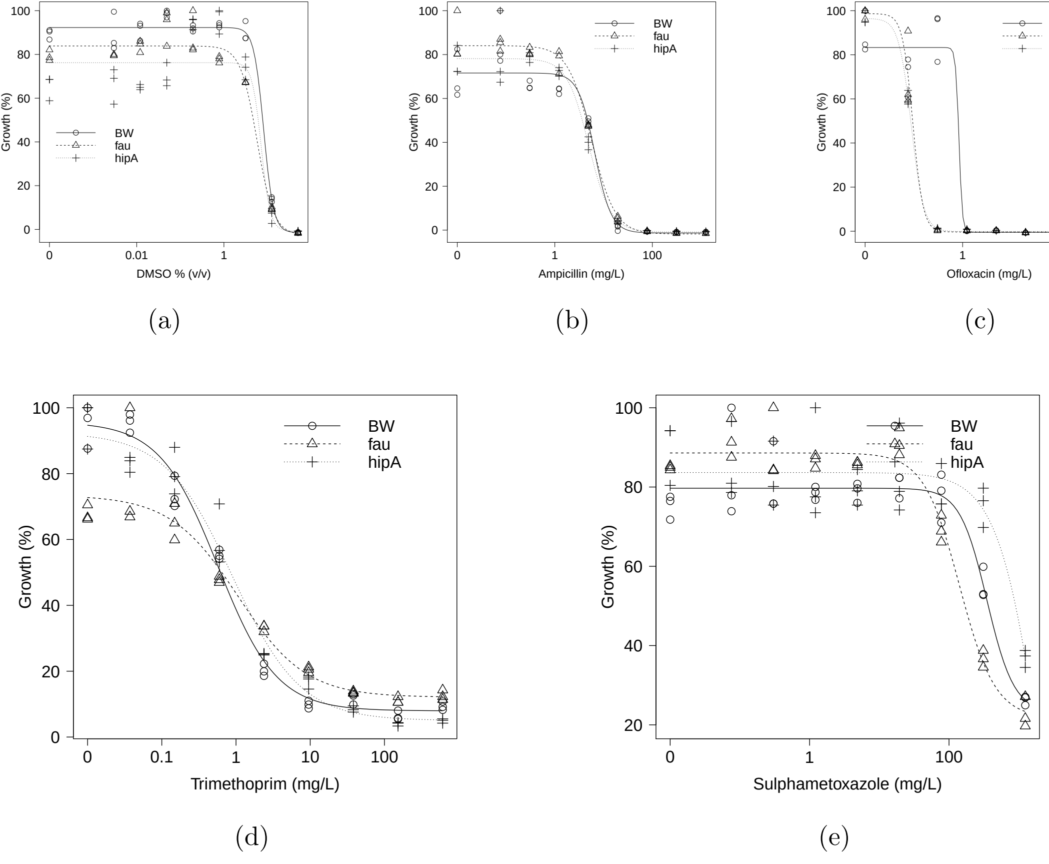




Supplementary Figure 1



Supplementary Figure 2

Supplementary Table 1: Time-dependent number of persisters in complex media. Number of persister in both BW25113 and folate mutant strains (JW2879-1) growing in batch cultures for three hours with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point for the folate mutant versus the parental strains are also included. Time points in hours are 0, 0.25, 0.5, 1, 2 , and 3.

Ampicillin								
BW25113								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	187660713	547767961	211753745	507128094	439552500	378772603	168187256	8.578
1	146076	247204	206626	179366	152424	186339	41642	5.27
2	71293	78382	79027	67072	55599	70274	9605	4.847
3	8891	11950	9330	11679	10193	10408	1369	4.017
4	3358	3002	3380	2130	3487	3071	557	3.487
5	816	1006	717	743	902	837	119	2.923
6	344	215	328	365	310	312	58.2	2.495
16	254	174	200	260	290	236	47.2	2.372
24	263	226	256	246	223	243	17.9	2.385
JW2879-1								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	21051628	35801723	56170735	36038338	37670957	37346676	12490772	7.572
1	125442	71759	130950	291193	335401	190949	115122	5.281
2	14388	11317	5891	9528	27219	13669	8175	4.136
3	2438	2411	4320	2618	2974	2952	797	3.47
4	1642	1418	997	1243	1008	1262	276	3.101
5	290	593	642	138	846	502	285	2.701
6	295	294	247	216	190	248	46.6	2.395
16	115	57	144	151	129	119	37.7	2.076
24	80	42	76	91	74	72.5	18.4	1.86
Ofloxacin								
BW25113								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	59767537	43068468	59827576	40166785	47534924	50073058	9257081	7.7
1	60414	60693	63551	86996	50570	64445	13530	4.809
2	242	303	365	131	401	289	107	2.46
3	59	35	68	116	120	79.7	37.4	1.902
4	16	48	30	43	7	28.8	17.5	1.459
5	18	11	14	35	37	23	12.2	1.361
6	5	20	7	25	50	21.6	17.9	1.334
16	18	14	23	29	30	22.8	6.79	1.357
24	15	16	47	37	6	24.2	17.3	1.384
JW2879-1								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	22298113	20176444	29232841	29075816	36491388	27454920	6461713	7.439
1	223	175	165	145	224	186	35.7	2.27
2	13	28	12	16	14	16.8	6.52	1.226
3	15	16	25	18	11	17	5.17	1.232
4	21	17	10	23	21	18.4	5.05	1.265
5	25	15	20	21	18	19.8	3.75	1.296
6	11	23	18	17	17	17.3	4.15	1.239
16	18	18	12	22	21	18.2	3.93	1.26
24	16	17	15	22	18	17.7	2.49	1.248

Supplementary Table 2: Time-dependent number of persisters in minimal media. Number of persister in both BW25113 and folate mutant strains (JW2879-1) growing in batch cultures for three hours with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point for the folate mutant versus the parental strains are also included. Time points in hours are 0, 0.25, 0.5, 1, 2 , and 3.

Ampicillin								
BW25113								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	10293805	10224679	10885937	9047462	11141440	10318665	809964	7.014
1	15721	13384	23705	26946	18953	19742	5586	4.295
2	1591	1616	975	1040	902	1225	349	3.088
3	116	137	139	145	41	116	43.3	2.063
4	13	11	13	21	14	14.7	3.77	1.166
5	2	3	2	1	1	1.76	0.75	0.245
6	3	1	2	2	2	2.08	0.43	0.318
16	2	3	1	2	3	1.97	0.76	0.295
24	2	2	3	2	2	2.02	0.45	0.305
JW2879-1								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	3663221	2769499	1760461	2914497	554138	2332363	1203153	6.368
1	10012	17361	18407	11204	11302	13657	3909	4.135
2	1092	411	1250	930	1516	1040	413	3.017
3	32	91	101	116	53	78.6	34.8	1.896
4	3	6	5	7	5	5.09	1.41	0.707
5	1	1	2	1	1	1.42	0.61	0.153
6	1	1	2	2	1	1.46	0.51	0.165
16	1	2	1	2	1	1.51	0.36	0.18
24	1	1	2	2	2	1.48	0.44	0.169
Ofloxacin								
BW25113								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	4101128	3179671	4127221	2675466	4002861	3617269	655999	6.558
1	6944	7789	6903	5291	8216	7029	1122	3.847
2	18	38	23	19	7	21.2	11.2	1.326
3	7	4	6	5	5	5.27	1.35	0.722
4	3	3	3	3	1	2.51	1.07	0.399
5	1	3	2	3	3	2.32	0.65	0.365
6	2	2	2	3	3	2.37	0.36	0.375
16	4	3	2	1	2	2.37	1.06	0.375
24	2	2	1	2	2	1.72	0.69	0.235
JW2879-1								
Time	1	2	3	4	5	Mean	Stdev	Log (mean)
0	2307456	3906190	1958115	3223489	5412309	3361512	1378591	6.527
1	24	13	11	15	17	15.9	5.05	1.2
2	1	1	1	1	2	1.22	0.25	0.088
3	1	2	2	2	1	1.57	0.31	0.195
4	2	1	2	2	2	1.56	0.33	0.194
5	1	1	1	1	1	1.23	0.23	0.089
6	2	1	2	2	1	1.54	0.5	0.187
16	1	1	1	2	1	1.35	0.28	0.131
24	2	1	1	1	1	1.22	0.41	0.088

Supplementary Table 3: The number of persisters to Ampicillin in complex media. Number of persister in both BW25113 and folate mutant strains (JW2879-1) growing in batch cultures for three hours with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point for the folate mutant versus the parental strains are also included. Time points in hours are 0, 0.25, 0.5, 1, 2 , and 3.

Complex media and Ampicillin						
	BW25113					
	0	0.25	0.5	1	2	3
1	3.83E+02	4.51E+02	9.80E+02	2.02E+03	8.77E+03	2.24E+04
2	4.60E+02	3.64E+02	8.30E+02	2.22E+03	7.77E+03	2.44E+04
3	6.00E+02	4.51E+02	1.00E+03	2.20E+03	7.54E+03	2.60E+04
4	5.30E+02	3.86E+02	9.86E+02	3.01E+03	8.54E+03	2.20E+03
5	5.32E+02	2.98E+02	9.87E+02	2.21E+03	8.54E+03	2.54E+04
Means	5.01E+02	3.90E+02	9.57E+02	2.33E+03	8.23E+03	2.01E+04
Std	8.25E+01	6.44E+01	7.11E+01	3.88E+02	5.41E+02	1.01E+04
Ratios	1.00E+00	7.78E-01	1.91E+00	4.66E+00	1.64E+01	4.00E+01
	JW2879-1					
	0	0.25	0.5	1	2	3
1	4.60E+01	6.10E+01	8.10E+01	2.31E+02	4.90E+02	1.02E+03
2	5.40E+01	7.20E+01	9.20E+01	1.80E+02	4.51E+02	1.08E+03
3	4.80E+01	7.40E+01	8.40E+01	1.60E+02	5.45E+02	1.09E+03
4	5.80E+01	6.40E+01	7.40E+01	2.10E+02	4.67E+02	9.82E+02
5	5.80E+01	6.90E+01	7.90E+01	1.90E+02	4.91E+02	1.06E+03
Means	5.28E+01	6.80E+01	8.20E+01	1.94E+02	4.89E+02	1.05E+03
Std	5.59E+00	5.43E+00	6.67E+00	2.74E+01	3.56E+01	4.57E+01
Ratios	1.00E+00	1.29E+00	1.55E+00	3.68E+00	9.26E+00	1.98E+01
p-values	1.98E-06	3.78E-06	3.43E-09	1.78E-06	1.01E-09	2.92E-03

Supplementary Table 4: The number of persisters to Ofloxacin in complex media. Number of persister in both BW25113 and folate mutant strains (JW2879-1) growing in batch cultures for three hours with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point for the folate mutant versus the parental strains are also included. Time points in hours are 0, 0.25, 0.5, 1, 2 , and 3.

Complex media and Ofloxacin						
	BW25113					
	0	0.25	0.5	1	2	3
1	2.60E+01	1.02E+02	1.50E+02	4.00E+02	1.95E+03	4.60E+03
2	1.70E+01	9.60E+01	1.32E+02	3.89E+02	1.93E+03	3.99E+03
3	1.60E+01	1.01E+02	1.89E+02	4.32E+02	1.99E+03	4.50E+03
4	1.64E+01	9.80E+01	2.01E+02	3.98E+02	1.88E+03	3.86E+03
5	1.84E+01	1.03E+02	1.98E+02	4.02E+02	1.99E+03	4.40E+03
Means	1.88E+01	9.97E+01	1.57E+02	4.07E+02	1.96E+03	4.36E+03
Std	4.15E+00	3.21E+00	2.91E+01	2.23E+01	2.65E+01	3.27E+02
Ratios	1.00E+00	5.31E+00	8.37E+00	2.17E+01	1.04E+02	2.33E+02
	JW2879-1					
	0	0.25	0.5	1	2	3
1	3.10E+00	3.50E+00	6.50E+00	1.50E+01	5.50E+01	1.38E+02
2	2.20E+00	3.89E+00	5.89E+00	1.64E+01	7.60E+01	1.70E+02
3	2.10E+00	3.45E+00	5.45E+00	1.25E+01	7.20E+01	1.35E+02
4	2.30E+00	4.21E+00	5.32E+00	1.36E+01	5.42E+01	1.42E+02
5	2.40E+00	4.12E+00	6.12E+00	1.74E+00	5.74E+01	1.46E+02
Means	2.42E+00	3.61E+00	5.95E+00	1.46E+01	6.77E+01	1.48E+02
Std	3.96E-01	2.41E-01	5.27E-01	1.95E+00	1.12E+01	1.95E+01
Ratios	1.00E+00	1.49E+00	2.46E+00	6.05E+00	2.80E+01	6.11E+01
p-values	2.24E-05	1.35E-12	2.03E-06	2.56E-11	2.60E-13	2.66E-09

Supplementary Table 5: The number of persisters to Ampicillin in minimal media. Number of persister in both BW25113 and folate mutant strains (JW2879-1) growing in batch cultures for three hours with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point for the folate mutant versus the parental strains are also included. Time points in hours are 0, 0.25, 0.5, 1, 2 , and 3.

Minimal media and Ampicillin						
	BW25113					
	0	0.25	0.5	1	2	3
1	2.51E+01	5.77E+00	1.10E+01	8.10E+00	3.00E-01	2.80E-01
2	3.51E+01	6.50E+00	2.00E+00	9.10E+00	6.33E-01	2.00E-01
3	5.00E+00	5.00E+00	2.12E+00	8.20E+00	3.20E-01	1.00E+00
4	2.71E+01	5.10E+00	1.30E+01	8.75E+00	6.45E-01	2.20E-01
5	3.50E+00	9.00E+00	3.00E+00	2.60E+00	7.33E-01	1.10E+00
Means	1.92E+01	6.27E+00	6.22E+00	7.35E+00	5.26E-01	5.60E-01
Std	1.41E+01	1.64E+00	5.33E+00	2.69E+00	2.01E-01	4.50E-01
Ratios	1.00E+00	3.27E-01	3.25E-01	3.84E-01	2.75E-02	2.92E-02
	JW2879-1					
	0	0.25	0.5	1	2	3
1	2.10E+01	7.25E+00	9.35E+00	3.35E+00	6.00E-01	3.75E+00
2	2.00E+01	8.20E+00	8.20E+00	6.00E+00	1.00E+00	4.85E+00
3	2.45E+01	8.30E+00	1.00E+01	1.00E+01	7.00E-01	3.62E+00
4	2.40E+01	7.65E+00	9.12E+00	5.70E+00	1.12E+00	5.00E+00
5	2.65E+01	1.08E+01	8.62E+00	1.21E+01	9.60E+00	3.96E+00
Means	2.32E+01	8.44E+00	9.06E+00	7.43E+00	2.60E+00	4.24E+00
Std	2.67E+00	1.39E+00	6.90E-01	3.54E+00	3.92E+00	6.43E-01
Ratios	1.00E+00	3.64E-01	3.91E-01	3.21E-01	1.12E-01	1.83E-01
p-values	5.49E-01	5.40E-02	2.73E-01	9.69E-01	2.70E-01	5.98E-06

Supplementary Table 6: The number of persisters to Ofloxacin in minimal media. Number of persister in both BW25113 and folate mutant strains (JW2879-1) growing in batch cultures for three hours with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point for the folate mutant versus the parental strains are also included.Time points in hours are 0, 0.25, 0.5, 1, 2 , and 3.

Minimal media and Ofloxacin						
	BW25113					
	0	0.25	0.5	1	2	3
1	2.25E+00	2.94E+00	3.85E-01	2.90E-02	6.50E-02	5.80E-02
2	4.90E+00	2.50E+00	1.20E+00	8.50E-02	6.00E-02	1.05E-01
3	3.00E+00	3.00E+00	2.00E+00	1.00E-01	1.00E-01	1.00E-01
4	3.90E+00	2.70E+00	1.40E+00	9.50E-02	5.90E-02	5.90E-02
5	2.90E+00	2.80E+00	2.32E+00	1.10E-01	1.20E-01	1.15E-01
Means	3.39E+00	2.79E+00	1.46E+00	8.38E-02	8.08E-02	8.74E-02
Std	1.03E+00	1.99E-01	7.51E-01	3.19E-02	2.77E-02	2.69E-02
Ratios	1.00E+00	8.23E-01	4.31E-01	2.47E-02	2.38E-02	2.58E-02
	JW2879-1					
	0	0.25	0.5	1	2	3
1	2.06E-01	8.10E-02	1.18E-02	4.50E-03	1.10E-03	1.60E-03
2	3.45E-01	1.35E-01	2.40E-02	1.40E-02	4.00E-03	1.70E-02
3	2.46E-01	9.10E-02	1.10E-01	5.00E-02	1.10E-02	3.00E-02
4	3.56E-01	1.46E-01	2.60E-02	1.50E-02	4.20E-03	1.40E-02
5	4.45E-01	2.35E-01	1.12E-01	4.90E-02	9.00E-03	2.90E-02
Means	3.20E-01	1.38E-01	5.68E-02	2.65E-02	5.86E-03	1.83E-02
Std	9.49E-02	6.11E-02	4.98E-02	2.14E-02	4.04E-03	1.17E-02
Ratios	1.00E+00	4.31E-01	1.78E-01	8.29E-02	1.83E-02	5.73E-02
p-values	1.63E-04	2.53E-09	3.12E-03	1.03E-02	3.26E-04	7.66E-04

Supplementary Table 7: The number of persisters in parental strain (BW25113) to Ampicillin in complex media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

BW25113 in complex media and Ampicillin				
	DMSO			
	0	1	2	3
1	2.58E+02	1.18E+03	9.30E+02	1.61E+03
2	3.00E+02	6.00E+02	1.50E+03	2.25E+03
3	4.00E+02	9.00E+02	9.00E+02	6.00E+02
4	3.34E+02	6.60E+02	1.35E+03	2.85E+03
5	3.40E+02	8.70E+02	1.05E+03	5.10E+02
Means	3.26E+03	8.42E+02	1.15E+03	1.56E+03
Std	5.27E+02	2.29E+02	2.66E+02	1.02E+03
Ratios	1.00E+00	2.58E+00	3.51E+00	4.79E+00
	SMX			
	0	1	2	3
1	1.80E+02	3.00E+02	7.70E+02	4.80E+02
2	2.50E+02	1.05E+03	6.00E+02	1.20E+03
3	2.00E+02	3.30E+02	8.00E+02	5.40E+02
4	2.10E+02	6.90E+02	6.30E+02	9.00E+02
5	1.90E+02	7.50E+02	5.70E+02	9.90E+02
Means	2.06E+02	6.24E+02	6.74E+02	8.22E+02
Std	2.70E+01	3.13E+02	1.04E+02	3.06E+02
Ratios	1.00E+00	3.03E+00	3.27E+00	3.99E+00
p-values	1.89E-03	2.45E-01	6.11E-03	1.58E-01
	TMP			
	0	1	2	3
1	4.38E+02	1.08E+02	1.00E+02	1.33E+02
2	4.00E+02	3.00E+02	3.00E+02	3.00E+02
3	3.38E+02	1.28E+02	1.25E+02	1.57E+02
4	4.30E+02	3.10E+02	3.27E+02	2.30E+02
5	3.20E+02	3.54E+02	1.43E+02	1.63E+02
Means	3.85E+02	2.40E+02	1.99E+02	1.96E+02
Std	5.36E+01	1.14E+02	1.06E+02	6.83E+01
Ratios	1.00E+00	6.23E-01	5.17E-01	5.10E-01
p-values	1.19E-01	5.23E-01	1.82E-02	6.98E-02

Supplementary Table 8:The number of persisters in parental strain (BW25113) to Ofloxacin in complex media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

BW25113 in complex media and Ofloxacin				
	DMSO			
	0	1	2	3
1	2.96E+01	1.88E+02	3.15E+02	1.14E+03
2	2.90E+01	1.97E+02	2.76E+02	1.29E+03
3	5.73E+01	1.43E+02	2.93E+02	8.09E+02
4	4.50E+01	8.02E+01	2.44E+02	1.10E+03
5	6.60E+01	1.00E+02	2.79E+02	1.00E+03
Means	4.54E+01	1.41E+02	2.81E+02	1.07E+03
Std	1.65E+01	5.17E+01	2.61E+01	1.79E+02
Ratios	1.00E+00	3.12E+00	6.20E+00	2.35E+01
	SMX			
	0	1	2	3
1	1.89E+02	2.04E+02	3.94E+02	2.30E+02
2	1.55E+02	2.23E+02	4.46E+02	1.87E+03
3	3.54E+01	2.74E+02	3.48E+02	1.26E+03
4	5.98E+01	1.55E+02	4.62E+02	3.80E+02
5	8.48E+01	3.52E+02	4.83E+02	8.35E+01
Means	1.05E+02	2.41E+02	4.27E+02	7.65E+02
Std	4.00E+01	7.53E+01	5.49E+01	1.50E+02
Ratios	1.00E+00	2.31E+00	4.08E+00	7.32E+00
p-values	8.25E-02	4.01E-02	6.86E-04	4.18E-01
	TMP			
	0	1	2	3
1	3.85E+01	4.85E+01	3.90E+01	4.31E+01
2	1.04E+02	2.87E+01	4.47E+01	4.26E+01
3	6.71E+01	4.53E+01	5.44E+01	4.73E+01
4	7.56E+01	6.14E+01	4.71E+01	4.48E+01
5	5.29E+01	2.98E+01	1.17E+01	5.00E+01
Means	6.76E+01	4.28E+01	3.94E+01	4.56E+01
Std	2.46E+01	1.37E+01	1.64E+01	3.09E+00
Ratios	1.00E+00	6.33E-01	5.83E-01	6.75E-01
p-values	4.06E-01	2.44E-04	2.16E-07	3.18E-02

Supplementary Table 9: The number of persisters in parental strain (BW25113) to Ampicillin in minimal media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

BW25113 in minimal media and Ampicillin				
	DMSO			
	0	1	2	3
1	6.25E+01	1.14E+01	1.26E+01	1.35E+01
2	4.65E+01	1.01E+01	1.10E+01	1.39E+01
3	3.16E+01	9.86E+00	7.98E+00	3.91E+00
4	3.94E+01	1.14E+01	1.77E+01	2.23E+01
5	4.32E+01	7.23E+00	1.03E+01	8.68E+00
Means	4.46E+01	1.00E+01	1.19E+01	1.25E+01
Std	1.14E+01	1.72E+00	3.62E+00	6.83E+00
Ratios	1.00E+00	2.24E-01	2.66E-01	2.79E-01
	SMX			
	0	1	2	3
1	3.88E+01	3.68E+01	8.57E+00	3.51E+00
2	3.24E+01	2.81E+01	9.60E+00	9.21E+00
3	3.33E+01	2.01E+01	1.01E+01	4.85E+00
4	7.31E+01	1.53E+01	8.85E+00	1.65E+00
5	6.30E+01	2.47E+01	9.56E+00	1.63E+01
Means	4.81E+01	2.50E+01	9.34E+00	6.44E+00
Std	1.87E+01	8.19E+00	6.31E-01	7.00E-01
Ratios	1.00E+00	5.20E-01	1.94E-01	1.34E-01
p-values	7.34E-01	3.93E-03	1.60E-01	2.19E-01
	TMP			
	0	1	2	3
1	7.92E+01	3.03E+01	3.90E+01	2.69E+01
2	1.13E+02	1.92E+01	1.62E+01	2.98E+01
3	1.25E+02	2.32E+01	2.34E+01	2.45E+01
4	1.07E+02	2.93E+01	2.58E+01	3.40E+01
5	1.55E+02	4.07E+01	2.68E+01	1.91E+01
Means	1.16E+02	2.85E+01	2.62E+01	2.69E+01
Std	2.77E+01	8.19E+00	8.23E+00	5.60E+00
Ratios	1.00E+00	2.46E-01	2.26E-01	2.32E-01
p-values	7.09E-04	1.12E-03	7.40E-03	6.53E-03

Supplementary Table 10: The number of persisters in parental strain (BW25113) to Ofloxacin in minimal media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

BW25113 in minimal media and Ofloxacin				
	DMSO			
	0	1	2	3
1	1.41E+00	2.15E+00	2.37E+00	1.88E+00
2	2.40E+00	1.44E+00	1.89E+00	1.85E+00
3	2.16E+00	9.62E-01	1.33E+00	1.96E+00
4	2.22E+00	1.46E+00	1.53E+00	1.85E+00
5	2.63E+00	1.93E+00	2.59E+00	1.98E+00
Means	2.16E+00	1.59E+00	1.94E+00	1.91E+00
Std	4.62E-01	4.65E-01	5.39E-01	6.30E-02
Ratios	1.00E+00	7.34E-01	8.98E-01	8.81E-01
	SMX			
	0	1	2	3
1	3.81E+00	1.42E+00	2.01E+00	2.01E+00
2	2.47E+00	1.72E+00	1.89E+00	2.02E+00
3	2.70E+00	1.55E+00	1.94E+00	2.00E+00
4	3.72E+00	1.83E+00	1.89E+00	2.00E+00
5	3.40E+00	1.71E+00	1.48E+00	1.99E+00
Means	3.22E+00	1.65E+00	1.84E+00	2.00E+00
Std	6.06E-01	1.61E-01	2.07E-01	1.31E-02
Ratios	1.00E+00	5.11E-01	5.71E-01	6.22E-01
p-values	1.45E-02	7.98E-01	6.97E-01	9.23E-03
	TMP			
	0	1	2	3
1	4.78E+00	1.78E+00	1.61E+00	1.33E+00
2	1.15E+01	1.94E+00	1.52E+00	9.32E-01
3	8.25E+00	2.02E+00	1.36E+00	1.21E+00
4	6.89E+00	1.76E+00	1.67E+00	1.45E+00
5	5.41E+00	1.72E+00	1.91E+00	1.50E+00
Means	7.36E+00	1.84E+00	1.61E+00	1.28E+00
Std	2.67E+00	1.29E-01	2.02E-01	2.26E-01
Ratios	1.00E+00	2.51E-01	2.19E-01	1.75E-01
p-values	8.02E-03	7.41E-02	1.54E-01	1.02E-04

Supplementary Table 11: The number of persisters in the hyper-persister strain ($\Delta hipA$) to Ampicillin in complex media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

$\Delta hipA$ in complex media and Ampicillin				
	DMSO			
	0	1	2	3
1	6.45E+03	6.23E+03	1.03E+04	3.11E+04
2	2.00E+03	7.50E+03	9.00E+03	5.40E+04
3	4.23E+03	6.86E+03	9.64E+03	4.25E+04
4	3.11E+03	7.18E+03	9.32E+03	4.83E+04
5	3.67E+03	7.02E+03	9.48E+03	4.54E+04
Means	3.89E+03	6.96E+03	9.54E+03	4.42E+04
Std	1.65E+03	4.73E+02	4.73E+02	8.51E+03
Ratios	1.00E+00	1.79E+00	2.45E+00	1.14E+01
	SMX			
	0	1	2	3
1	2.70E+03	3.27E+03	1.42E+04	1.61E+04
2	2.33E+03	6.70E+03	8.63E+03	4.80E+04
3	4.00E+03	7.50E+03	1.35E+04	1.50E+05
4	3.01E+03	5.82E+03	1.21E+04	7.14E+04
5	3.11E+03	6.67E+03	1.14E+04	8.98E+04
Means	3.03E+03	5.99E+03	1.20E+04	7.50E+04
Std	6.23E+02	1.63E+03	2.16E+03	5.02E+04
Ratios	1.00E+00	1.98E+00	3.95E+00	2.48E+01
p-values	3.06E-01	2.40E-01	4.04E-02	2.13E-01
	TMP			
	0	1	2	3
1	1.37E+03	1.90E+02	1.07E+03	1.10E+03
2	2.85E+03	7.67E+02	4.50E+02	1.63E+03
3	4.00E+03	2.00E+03	1.50E+03	1.00E+03
4	2.74E+03	9.86E+02	1.01E+03	1.24E+03
5	3.20E+03	1.25E+03	9.86E+02	1.29E+03
Means	2.83E+03	1.04E+03	1.00E+03	1.25E+03
Std	9.54E+02	6.64E+02	3.73E+02	2.38E+02
Ratios	1.00E+00	2.67E-01	2.58E-01	3.22E-01
p-values	2.49E-01	3.02E-03	2.12E-06	5.48E-06

Supplementary Table 12: The number of persisters in the hyper-persister strain ($\Delta hipA$) to Ofloxacin in complex media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting OD_{600} = 0.1. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

$\Delta hipA$ in complex media and Ofloxacin				
	DMSO			
	0	1	2	3
1	8.39E+01	1.03E+02	5.58E+03	6.50E+03
2	1.22E+02	1.54E+02	6.37E+03	4.83E+03
3	1.01E+02	1.22E+02	8.34E+03	1.40E+04
4	1.31E+02	1.21E+02	1.76E+03	1.21E+04
5	8.38E+01	1.26E+02	5.21E+03	8.71E+03
Means	1.04E+02	1.25E+02	5.45E+03	9.22E+03
Std	2.15E+01	1.85E+01	2.39E+03	3.80E+03
Ratios	1.00E+00	1.20E+00	5.23E+01	8.84E+01
	SMX			
	0	1	2	3
1	3.18E+02	2.95E+02	1.34E+03	1.50E+04
2	2.88E+02	2.87E+02	1.26E+03	1.22E+04
3	4.12E+02	4.16E+02	1.01E+03	1.72E+04
4	3.39E+02	3.39E+02	5.66E+02	8.12E+03
5	4.02E+02	3.04E+02	5.56E+02	7.16E+03
Means	3.52E+02	3.28E+02	9.47E+02	1.19E+04
Std	5.36E+01	5.29E+01	3.72E+02	4.32E+03
Ratios	1.00E+00	9.33E-01	2.69E+00	3.40E+01
p-values	1.18E-05	4.01E-05	3.17E-03	3.22E-01
	TMP			
	0	1	2	3
1	1.54E+02	3.82E+01	1.89E+01	1.34E+01
2	7.93E+01	4.51E+01	4.35E+01	1.64E+01
3	1.21E+02	3.90E+01	2.60E+01	1.79E+01
4	4.92E+01	4.36E+01	2.76E+01	1.27E+01
5	2.00E+02	3.76E+01	1.47E+01	1.60E+01
Means	1.21E+02	4.07E+01	2.61E+01	1.53E+01
Std	5.97E+01	3.41E+00	1.10E+01	2.15E+00
Ratios	1.00E+00	3.37E-01	2.17E-01	1.27E-01
p-values	5.78E-01	8.23E-06	9.65E-04	6.32E-04

Supplementary Table 13: The number of persisters in the hyper-persister strain (Δ*hipA*) to Ampicillin in minimal media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

Δ*hipA* in minimal media and Ampicillin				
	DMSO			
	0	1	2	3
1	9.14E+02	7.80E+02	7.44E+02	7.23E+02
2	1.04E+03	7.43E+02	7.05E+02	7.45E+02
3	1.02E+03	9.79E+02	6.07E+02	5.20E+02
4	1.01E+03	1.04E+03	8.05E+02	5.65E+02
5	1.21E+03	7.24E+02	8.17E+02	8.33E+02
Means	1.04E+03	8.53E+02	7.36E+02	6.77E+02
Std	1.06E+02	1.46E+02	8.51E+01	1.31E+02
Ratios	1.00E+00	8.22E-01	7.09E-01	6.52E-01
	SMX			
	0	1	2	3
1	1.44E+03	7.80E+02	7.77E+02	7.40E+02
2	1.75E+03	1.14E+03	8.15E+02	6.16E+02
3	1.78E+03	7.69E+02	7.67E+02	6.68E+02
4	1.16E+03	5.96E+02	7.06E+02	7.59E+02
5	1.39E+03	1.21E+03	8.39E+02	4.45E+02
Means	1.50E+03	8.98E+02	7.80E+02	6.46E+02
Std	2.63E+02	2.62E+02	5.09E+01	1.26E+02
Ratios	1.00E+00	5.97E-01	5.19E-01	4.29E-01
p-values	6.26E-03	7.48E-01	3.43E-01	7.07E-01
	TMP			
	0	1	2	3
1	9.71E+02	3.65E+02	3.94E+02	2.68E+02
2	6.78E+02	3.82E+02	4.81E+02	2.72E+02
3	1.54E+03	4.89E+02	4.16E+02	4.79E+02
4	1.43E+03	7.00E+02	1.59E+02	3.71E+02
5	5.45E+02	7.00E+02	4.67E+02	3.81E+02
Means	1.03E+03	5.27E+02	3.84E+02	3.54E+02
Std	4.43E+02	1.65E+02	1.30E+02	8.77E+01
Ratios	1.00E+00	5.10E-01	3.71E-01	3.43E-01
p-values	9.82E-01	1.06E-02	9.79E-04	1.78E-03

Supplementary Table 14: The number of persisters in the hyper-persister strain ($\Delta hipA$) to Ofloxacin in minimal media after pre-incubating with antifolates. Number of persister in batch cultures pre-incubated for upto three hours in antifolates with an starting $OD_{600} = 0.1$. Data in cfu/mL. Means and standard deviations (Std) are presented for five different experiments (n=5). Ratios of number of persisters are listed for each time point against time zero. The p-values of T-tests for data in each time point of the pre-incubation in either SMX or TMP versus the control in DMSO are also included. Time points in hours are 0, 1, 2, and 3.

$\Delta hipA$ in minimal media and Ofloxacin				
	DMSO			
	0	1	2	3
1	1.32E+01	3.67E+01	4.43E+01	4.97E+01
2	1.27E+01	5.52E+01	6.34E+01	3.82E+01
3	1.07E+01	4.89E+01	2.87E+01	4.62E+01
4	1.23E+01	1.17E+01	4.60E+01	4.87E+01
5	1.10E+01	3.82E+01	1.99E+01	4.01E+01
Means	1.20E+01	3.81E+01	4.05E+01	4.46E+01
Std	1.10E+00	1.67E+01	1.68E+01	5.16E+00
Ratios	1.00E+00	3.18E+00	3.38E+00	3.72E+00
	SMX			
	0	1	2	3
1	1.18E+01	8.48E+00	3.25E+00	1.97E+00
2	1.19E+01	1.21E+01	4.73E+00	2.47E+00
3	1.37E+01	1.54E+01	2.59E+00	2.07E+00
4	1.47E+01	1.18E+01	5.07E+00	2.34E+00
5	1.28E+01	1.31E+01	2.48E+00	2.29E+00
Means	1.30E+01	1.22E+01	3.62E+00	2.23E+00
Std	1.25E+00	2.50E+00	1.21E+00	2.04E-01
Ratios	1.00E+00	9.39E-01	2.80E-01	1.72E-01
p-values	2.28E-01	8.71E-03	1.22E-03	8.02E-08
	TMP			
	0	1	2	3
1	1.60E+02	3.62E+01	2.09E+01	9.82E+00
2	1.34E+02	6.76E+01	2.64E+01	7.89E+00
3	9.09E+01	2.85E+01	6.68E+00	1.27E+01
4	1.92E+02	4.05E+01	1.71E+01	7.71E+00
5	1.20E+02	3.85E+01	2.79E+01	9.38E+00
Means	1.39E+02	4.22E+01	1.98E+01	9.51E+00
Std	3.87E+01	1.49E+01	8.53E+00	2.02E+00
Ratios	1.00E+00	3.03E-01	1.42E-01	6.82E-02
p-values	7.96E-05	6.92E-01	3.99E-02	6.04E-07

Supplementary Table 15: Ranked data for Principal Components Analysis. The six different variables were ranked as follows: Strain (1=BW25113, 2=Δ*hipA*), Time in hours (0,1,2,3), Antifolate (1=DMSO, 2=SMX, 3=TMP), Media (1=minimal media, 2=complex media), Antibiotic (1=Ampicillin, 2=Ofloxacin), and Persisters (the ratio of cfu/mL at each time point in antifolates over the cfu/mL at time zero).

Strain	Time	Antifolate	Media	Antibiotic	Persisters	Strain	Time	Antifolate	Media	Antibiotic	Persisters
1	0	1	1	1	1	1	0	1	2	1	1
1	1	1	1	1	0.224	1	1	1	2	1	2.58
1	2	1	1	1	0.266	1	2	1	2	1	3.51
1	3	1	1	1	0.279	1	3	1	2	1	4.79
1	0	2	1	1	1	1	0	2	2	1	1
1	1	2	1	1	0.00393	1	1	2	2	1	3.03
1	2	2	1	1	0.16	1	2	2	2	1	3.27
1	3	2	1	1	0.219	1	3	2	2	1	3.99
1	0	3	1	1	1	1	0	3	2	1	1
1	1	3	1	1	0.246	1	1	3	2	1	0.623
1	2	3	1	1	0.226	1	2	3	2	1	0.517
1	3	3	1	1	0.232	1	3	3	2	1	0.51
1	0	1	1	2	1	1	0	1	2	2	1
1	1	1	1	2	0.734	1	1	1	2	2	3.12
1	2	1	1	2	0.898	1	2	1	2	2	6.2
1	3	1	1	2	0.881	1	3	1	2	2	2.35
1	0	2	1	2	1	1	0	2	2	2	1
1	1	2	1	2	0.511	1	1	2	2	2	2.31
1	2	2	1	2	0.571	1	2	2	2	2	4.08
1	3	2	1	2	0.622	1	3	2	2	2	7.32
1	0	3	1	2	1	1	0	3	2	2	1
1	1	3	1	2	0.251	1	1	3	2	2	0.633
1	2	3	1	2	0.219	1	2	3	2	2	0.583
1	3	3	1	2	0.175	1	3	3	2	2	0.657
2	0	1	1	1	1	2	0	1	2	1	1
2	1	1	1	1	0.822	2	1	1	2	1	1.79
2	2	1	1	1	0.709	2	2	1	2	1	2.45
2	3	1	1	1	0.652	2	3	1	2	1	1.14
2	0	2	1	1	1	2	0	2	2	1	1
2	1	2	1	1	0.597	2	1	2	2	1	1.98
2	2	2	1	1	0.519	2	2	2	2	1	3.95
2	3	2	1	1	0.429	2	3	2	2	1	2.48
2	0	3	1	1	1	2	0	3	2	1	1
2	1	3	1	1	0.51	2	1	3	2	1	0.267
2	2	3	1	1	0.371	2	2	3	2	1	0.258
2	3	3	1	1	0.343	2	3	3	2	1	0.322
2	0	1	1	2	1	2	0	1	2	2	1
2	1	1	1	2	3.18	2	1	1	2	2	1.2
2	2	1	1	2	3.38	2	2	1	2	2	5.23
2	3	1	1	2	3.72	2	3	1	2	2	8.84
2	0	2	1	2	1	2	0	2	2	2	1
2	1	2	1	2	0.939	2	1	2	2	2	0.933
2	2	2	1	2	0.28	2	2	2	2	2	2.69
2	3	2	1	2	0.172	2	3	2	2	2	3.4
2	0	3	1	2	1	2	0	3	2	2	1
2	1	3	1	2	0.303	2	1	3	2	2	0.337
2	2	3	1	2	0.142	2	2	3	2	2	0.217
2	3	3	1	2	0.0682	2	3	3	2	2	0.127

Supplementary Table 16: Primers for RT-PCR

Pathway	EC / TC number	Name	Enzyme	LEFT	RIGHT
Pteroate	3.5.4.16	folE	GTP cyclohydrolase	TCTGGATTACGCCAATTTCC	TTCACAGGTGCTGGTCAGAG
Pteroate	3.6.1.67	nudB	Dihydroneopterin triphosphate pyrophosphohydrolase	GTCAGCGCACGGTAGAGTTT	GAAGCGCAAGACAGAACCAT
Pteroate	4.1.2.25	folB	Dihydroneopterin aldolase	TGTTAGCACGCTTCAACTCG	TTGCCACGCTCAATGATTAC
Pteroate	2.7.6.3	folK	6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase	CGCAAGATCAACCCGATTAC	CTTGCTGCAATTCAATACGC
Pteroate	2.5.1.15	folP	Dihydropteroate synthase	TCACTGGACCTTAGCCATCC	ACCGCATCTATCAGCGAGTT
Pteroate	2.5.1.54	aroF	2-dehydro-3-deoxyphosphoheptonate aldolase	GAAGCCCAGATTGCTGACTC	TGAATGGAACAAGGACCACA
Pteroate	2.5.1.54	aroH	2-dehydro-3-deoxyphosphoheptonate aldolase	ATCGGGTAAATCACGGTCTG	CGGTCACCATATCGAGGAAC
Pteroate	2.5.1.54	aroG	2-dehydro-3-deoxyphosphoheptonate aldolase	GATGGATGTTTGTGCTGACG	CTGATTGCCTTCCACCAGAT
Shikimate	4.2.3.4	aroB	3-dehydroquinate synthase	CTGTATTCGCCGTTGTTGTG	CCAAAGGTGTGTCCCAGATT
Shikimate	4.2.1.10	aroD	3-dehydroquinate dehydratase	AATTCTCCGTGAGACCATGC	GCAATATAAGCCTCGGTGGA
Shikimate	1.1.1.282	aroE	shikimate dehydrogenase	GCTCATTCATCCAGGCATTT	CCATCAGCATTACGCTTTGA
Shikimate	1.1.1.25	ydiB	shikimate dehydrogenase	AAACGATGGTGCTGTTAGGG	TCATCCCGACGGTTAAAGAG
Shikimate	2.7.1.71	aroK (aroL)	shikimate kinase	AACGACCATCGAAAAGCAAC	AACGCTTCCAGAACTTCACG
Shikimate	2.5.1.19	aroA	3-phosphoshikimate-1-carboxyvinyltransferase	TATTAATCTGCCCGGTTCCA	ATCGCTATCCAGCAGATTGG
Shikimate	4.2.3.5	aroC	chorismate synthase	AAAGACGGTTTCCAGAGCAA	TAATGCTGGAGGTCGGTTTC
pABA	2.6.1.85	pabBpabA	4-amino-4-deoxychorismate synthase	GGCGCAACTACCAGAACAGT	TTCCATAGCCCGTACTTTCG
pABA	4.1.3.38	pabC	aminodeoxychorismate lyase	GACTGGATCAGGCAGGTGTT	TTCCAGAGAGGCTTGCACTT
Folate	6.3.2.12 6.3.2.17	folC	dihydrofolate synthase and FPGS	ATGAAAGCGCTACCGAAAAA	ATCAACCACGCTTTTCAACC
Folate	1.5.1.3	folA (folM)	dihydrofolate reductase	CCATACCTGGGAATCAATCG	ACCGACTTCACCCACGTTAC
Folate	2.1.2.1	glyA	shmt	GACTCACGGTTCTCCGGTTA	TTTTCCAGATCGGCGTAGTC
Folate	N/A	gcvH	gcv system	GGCGATATGGTGTTTGTTGA	ATGTCTGACGCCGCTTTTAC
Folate	1.4.4.2	gcvP	gcv system	GCTGGAGCGTAAAGATCTGG	AGGTGATTGGGATCATCTCG
Folate	2.1.2.10	gcvT	gcv system	AAGATTTCTTCCGCCTCGTT	ACGAACGGTAATTTCGATGC
Folate	1.8.1.4	lpdA	gcv system	TCACCAAGCGTATCAGCAAG	TTGCCTTCCATCGTCACATA
Folate	1.5.1.5 3.5.4.9	folD	methylenetetrahydrofolate dehydrogenase & cyclohydrolase	TCTATTGATCGTTGCCGTTG	ATTTTCCAGACGGTTGATGC
Folate	6.3.3.2	ygfA (fau)	5-formyltetrahydrofolate cycloligase	CCTCTCTTTTGATGGCGAAC	GGCACTAAAGGGATGCAAAA
Folate	1.5.1.20	metF	5-MTHFR	GGCGGATTTGCTTAATCTGA	CGAAAACGCAGGTAGCTTTC
Folate	2.1.1.13	metH	methionine synthase	ATGGATATGGGGATCGTCAA	CGCGACGATTAAGAATCACA
Transport	2.A.39.1.1	codB	cytosine transporter	CACTCTCGGAACCGGTCTTA	CGCCAATGTAACCGAGAAAT
Transport	2.A.21.2	putP	proline:Na+ symporter	GTGCTGTTCTCGGTGATGTG	CCGAACTGTTTCCAGACGAT
Transport	2.A.3.7.1	gadC	glutamic acid:4-aminobutyrate antiporter	TTTATTCCCGTGGGACTTTG	ATCTCGGCCCCAGAGTATTT
Transport	2.A.8.1.5	dsdX	D-serine transporter	GCAGATATCGGTTCGGTGAT	GCAGTCGTTGACCCAGAAAT
Transport	2.A.1.40	adeQ	adenine transporter	GGTTGGCGTTATGTTCCTGT	CAGCACGCCAACAAAGATTA
Transport	4.B.1.1.1	pnuC	nicotinamide riboside transporter	CGACAAACCAGTCAGAACGA	TCAGACCAATCGAAACAACG
Transport	2.A.41.1.1	nupC	nucleoside:H+ symporter NupC	TCCATCGTTGGTGCATACAT	GATCAGCGACAGCACGATAA
Transport	2.A.1.10.1	nupG	nucleoside:H+ symporter NupG	CGCATTCGACTTCTTCAACA	ATCAGGAACATCCCTTGTGC
Transport	2.A.21.1.1	panF	pantothenate:Na+ symporter	CTTCCTGCAGTCTGGCTTTC	GGCAAACAGCATATCGTTCA
Transport	2.A.3.1.3	aroP	aromatic amino acid:H+ symporter AroP	GTTCGTTAGCCGTTCTGCTC	GCCACAAAGGTATCGCCTAA
Transport	2.A.14.1.2	glcA	glycolate / lactate:H+ symporter	AGGTACAGGCGTGATGTTCC	GAACCAAACAGGGCGTTAGA

Supplementary Table 16 continuation: Primers for RT-PCR

Pathway	EC / TC number	Name	Enzyme	LEFT	RIGHT
Transport	2.A.1.4.1	uhpT	hexose-6-phosphate:phosphate antiporter	ACCTACGGGTTGAGCATGAC	GTCGGCGTAGTAGGAAACCA
Transport	2.A.3.1.7	cycA	serine / alanine / glycine / cycloserine:H+symporter	GGGCTGACGTTCTCGTGTAT	GCGGAAACGGTTGTAATCAT
Transport	2.A.47.3.2	citT	citrate:succinate antiporter	CGGGTGATGAACTGAAAACC	TCACTGCCAAATACCCACAA
Transport	2.A.1.6.6	shiA	shikimate:H+ symporter	GCTATAGTGGCGCTGGAGTC	GTTCCCGGCAAAGTAAGTGA
Control	1.3.1.76	cysG	control: Siroheme synthase	TCGTCGCATCTTCTGTAACG	AGAGGAGACCGCTACCATGA
Control	2.A1.27.1	hcaT	control: Probable 3-phenylpropionic acid transporter	GTGGTGCAAATTCTGCATTG	CGCCTGTAAACGGATGACTT
Control	2.A.8.1.2	idnT	control: Gnt-II system L-idonate transporter	GTGGGTTTTGTCCTGCTGTT	ACAGAGAGCGCTGCTACCAT
Lipogenesis	6.4.1.2	accA	acetyl-CoA carboxyltransferase, subunit	GGTTAGCCGTCAGGATGAGA	ATCGGCGAAGATTTTACGTG
Lipogenesis	6.4.1.2	accB	biotinylated biotin-carboxyl carrier protein carboxybiotin-carboxyl-carrier protein biotin carboxyl carrier protein	AATCAGGCATCTCCGAACTG	CTTGTTGCATCACAGGGAAA
Lipogenesis	6.3.4.14	accC	AccC [component of biotin carboxylase]	GCGTACGTTGGGAGTCTCAT	CGGTTTTCACCGTAGCAAAT
Lipogenesis	6.4.1.2	accD	acetyl-CoA carboxyltransferase, subunit	CCTGAAGGGGTGTGGACTAA	TGGTCACACTTCGGACAGAC
Lipogenesis	2.3.1.85, 2.3.1.86, 4.2.1.59	fabA	FabA [component of -hydroxyacyl-ACP dehydratase/isomerase]	AACGGGTGGTAACTTCGACA	TAACCGGATCGCCAATAAAG
Lipogenesis	2.3.1.41	fabB	FabB [component of KASI]	TCCGCACACTGTATCGGTAA	CATTTCCCAGCACAGCTCTT
Lipogenesis	2.3.1.39, 2.3.1.86	fabD	malonyl-CoA-ACP transacylase	CGCAATTTGCATTTGTGTTC	CAGCAAACGTTTCTTCGACA
Lipogenesis	2.3.1.41, 2.3.1.85, 2.3.1.86	fabF	FabF [component of KASII]	CGGACTGATCGAAGAAAACC	CTGCCACCATGTTCACAATC
Lipogenesis	1.1.1.100	fabG	3-oxoacyl-[acyl-carrier-protein] reductase subunit	TGTTTTCCGTCTGTCAAAAGC	CATTTCCCATGGTACCAACC
Lipogenesis	2.3.1.38, 2.3.1.85, 2.3.1.86	fabH	FabH [component of KASIII]	GGGCATTGAGAAAGACCAGA	GCCCAACATGCTTTGAATCT
Lipogenesis	1.3.1.9	fabI	enoyl-[acyl-carrier-protein] reductase subunit	GCTGAACTGGGGAAAGTTTG	CGGCGTTAACATAGTCACCA
Lipogenesis	4.2.1.59	fabZ	FabZ [component of 3-hydroxy-acyl-[acyl-carrier-protein] dehydratase]	TCAATGAGCCATTCTTCCAG	AACGCCAGAATACCTGTTGC
Oxphox	1.10.3.14	cydX	cytochrome d (bd-I) ubiquinol oxidase subunit X	TGGATTCTGGGAACGCTTCT	TCTTCTTGACCGGCTTTGC

Relative Gene expression BW25113

	0H	0H	0H	0.25H	0.25H	0.25H	0.5H	0.5H	0.5H	1H	1H	1H	2H	2H	2H
folE	9.144675	10.2175	8.07185	44.45211	66.97589	21.92833	12.720155	10.84039	14.59992	6.024225	4.78223	7.26622	0.99996	194.01193	97.505945
nudB	10.561335	12.4564	8.66627	182.47689	267.31897	97.6348	79.16679	112.77424	45.55934	42.75366	44.54839	40.95893	1.00352	27900.735	13950.869
folB	2.661545	3.34476	1.97833	7.937515	9.68894	6.18609	7.07548	6.84668	7.30428	4.81313	4.54714	5.07912	1.00201	540.11508	270.55855
folK	5.905655	10.08321	1.7281	32.286755	54.48965	10.08386	19.92579	24.89182	14.95976	10.315805	7.39293	13.23868	1.00157	560.93168	280.96663
folP	1.657095	2.47971	0.83448	10.5371	15.36613	5.70807	4.479505	5.6999	3.25911	2.136175	1.13477	3.13758	1.0006	14.72163	7.861115
aroF	30.13119	37.94379	22.31859	445.41884	723.58134	167.25633	80.02288	92.19591	67.84985	20.62809	24.24037	17.01581	1.00154	134.25875	67.630145
aroH	2.847495	4.34695	1.34804	31.021415	56.45801	5.58482	5.29174	6.09191	4.49157	1.608	2.11288	1.10312	1.0004	12.05563	6.528015
aroG	15.48088	7.60401	23.35775	10.120975	11.98808	8.25387	7.140865	1.87864	12.40309	2.019055	1.14639	2.89172	1.00087	34.24653	17.6237
aroB	12.02445	19.22162	4.82728	383.88442	653.27626	114.49258	61.021925	72.24937	49.79448	22.68587	23.57503	21.79671	1.00256	989.98742	495.49499
aroD	0.51624	0.39951	0.63297	0.18429	0.08319	0.28539	0.17202	0.04141	0.30263	0.129625	0.04051	0.21874	0.99862	0.01839	0.508505
aroE	25.57353	34.27954	16.86752	109.47944	172.47998	46.47889	52.292855	54.21134	50.37437	18.006845	17.99536	18.01833	1.00274	2979.8545	1490.4286
ydiB	0.479545	0.38448	0.57461	0.12216	0.01434	0.22998	0.08769	0.00357	0.17181	0.01069	0.00151	0.01987	0.99792	0.00006	0.49899
aroK(aroL)	91.163545	135.86932	46.45777	4190.7002	7368.2562	1013.1442	733.09986	1047.1859	419.01378	207.22044	366.05146	48.38941	1.00424	161758.94	80879.974
aroA	7.5285	11.80355	3.25345	9.04038	14.93966	3.1411	3.01088	3.30273	2.71903	2.652345	1.81881	3.48588	1.00103	15.2027	8.101865
aroC	2.42583	3.38421	1.46745	17.08819	27.93392	6.24246	6.49109	6.88303	6.09915	4.61915	4.65421	4.58409	1.00072	62.61945	31.810085
pabBpabA	1.426235	1.83481	1.01766	3.04126	4.35	1.73252	1.112055	0.74175	1.48236	0.50403	0.49097	0.51709	1.00001	1.73056	1.365285
pabC	4.42432	6.21253	2.63611	85.07338	154.91945	15.22731	7.653155	7.34199	7.96432	2.62361	2.43352	2.8137	1.00095	102.08047	51.54071
folC	1.63338	1.77785	1.48891	20.27686	30.12042	10.4333	5.77724	3.58925	7.96523	2.364265	2.49021	2.23832	1.00016	15.61466	8.30741
folA(folM)	5.615435	7.94487	3.286	35.65792	55.62122	15.69462	27.995965	33.91267	22.07926	8.391675	10.95998	5.82337	1.00178	414.61962	207.8107
glyA	14.838485	21.67411	8.00286	34.57464	60.7217	8.42758	24.53242	38.62525	10.43959	14.650415	25.95596	3.34487	1.00199	5341.4005	2671.2012
gcvH	3.16662	3.40263	2.93061	1.12738	1.05555	1.19921	0.44108	0.34727	0.53489	0.61118	0.18044	1.04192	1.00037	2.60841	1.80439
gcvP	1.116535	1.14466	1.08841	0.3385	0.18545	0.49155	0.16774	0.09668	0.2388	0.33564	0.04673	0.62455	0.99929	0.0901	0.544695
gcvT	1.97759	2.16201	1.79317	0.881345	0.65161	1.11108	0.391435	0.308	0.47487	0.259555	0.11847	0.40064	0.99972	0.38943	0.694575
lpdA	21.726115	20.02349	23.42874	27125.794	51367.628	2883.9602	4532.562	7085.4194	1979.7047	1086.4594	2077.794	95.12476	1.00295	66388.326	33194.665
folD	12.647805	13.12487	12.17074	85.208535	154.19467	16.2224	32.89442	54.14695	11.64189	25.302165	26.87719	23.72714	1.00174	6966.8381	3483.9199
ygfA(fau)	57.95739	63.53668	52.3781	1219.0797	2277.2254	160.93407	229.24736	296.22766	162.26705	60.91292	120.49573	1.33011	1.00332	10485.983	5243.4932
metF	0.765685	0.81718	0.71419	2.69431	2.76269	2.62593	1.073305	0.91674	1.22987	0.488115	0.60774	0.36849	1.00044	3.78082	2.39063
metH	0.382925	0.48166	0.28419	0.516965	0.31747	0.71646	0.297145	0.09327	0.50102	0.366125	0.07865	0.6536	0.9993	0.16205	0.580675
codB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
putP	3.37969	3.58209	3.17729	40.269805	62.70533	17.83428	6.714855	8.72817	4.70154	2.07238	3.34642	0.79834	1.00099	59.11982	30.060405
gadC	45.064885	72.57586	17.55391	0.0069	0.00303	0.01077	0.049345	0.00775	0.09094	0.000735	0.00006	0.00141	0.10442	0.00055	0.052485
dsdX	0.54957	0.46704	0.6321	0.01518	0.0011	0.02926	0.10493	0.00784	0.20202	0.004675	0.00013	0.00922	0.31167	0.00192	0.156795
adeQ	0.859715	0.46293	1.2565	0.239045	0.00382	0.47427	0.39079	0.02526	0.75632	0.004685	0.00076	0.00861	2.00766	0.01454	1.0111
pnuC	2.511905	0.73027	4.29354	0.124195	0.05193	0.19646	0.31194	0.09393	0.52995	0.009515	0.0179	0.00113	0.42715	0.05663	0.24189
nupC	1.185085	1.04861	1.32156	0.32361	0.32118	0.32604	0.741955	0.61524	0.86867	1.56146	0.71057	2.41235	1.88578	1.00788	1.44683
nupG	1.057635	0.85118	1.26409	0.21522	0.09566	0.33478	0.162275	0.15658	0.16797	0.038595	0.03719	0.04	1.38709	0.20482	0.795955
panF	1.63384	1.1793	2.08838	37.287555	38.71557	35.85954	4.32776	6.91999	1.73553	12.12324	22.10677	2.13971	11.74694	44.33946	28.0432
aroP	1.27593	1.05755	1.49431	1.58138	1.96095	1.20181	2.1983	1.79501	2.60159	1.52608	2.88066	0.1715	1.09334	3.72996	2.41165
glcA	0.69173	0.49562	0.88784	0.052805	0.0043	0.10131	0.222985	0.03658	0.40939	0.00204	0.00299	0.00109	0.45959	0.03834	0.248965
uhpT	0.412055	0.27857	0.54554	0.221195	0.02561	0.41678	0.38966	0.03445	0.74487	0.00437	0.00053	0.00821	0.50884	0.00804	0.25844

cycA	2.85968	3.19984	2.51952	6.63327	8.6292	4.63734	5.70624	4.50641	6.90607	25.5129	49.23326	1.79254	5.6855	69.15694	37.42122
citT	0.85046	0.45153	1.24939	0.06892	0.0038	0.13404	0.092645	0.02371	0.16158	0.001645	0.00066	0.00263	0.32419	0.0101	0.167145
shiA	4.649155	4.0415	5.25681	2.807625	2.92234	2.69291	1.94618	1.33506	2.5573	2.1402	3.86584	0.41456	1.97207	17.57073	9.7714
cysG	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
hcaT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
idnT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
accA	10.0124	12.08004	7.94476	982.10499	1574.3515	389.85852	86.50489	88.37858	84.6312	4073.6034	8117.5061	29.70071	105.55337	6766.6534	3436.1034
accB	45.66983	74.48682	16.85284	57736.375	100121.83	15350.924	812.82187	1069.5765	556.0672	289622.98	578845.89	400.06816	213.32127	26442.569	13327.945
accC	2.266245	2.5161	2.01639	41.66124	40.78021	42.54227	12.28659	11.1305	13.44268	202.85683	398.60796	7.1057	65.10072	565.10525	315.10299
accD	6.580245	7.92599	5.2345	100.91522	170.25429	31.57615	15.67808	13.75495	17.60121	269.99764	535.70169	4.29358	49.69134	548.89036	299.29085
fabA	39.484275	58.86887	20.09968	41805.414	79546.09	4064.7374	139.41276	274.77044	4.05508	127812.27	255488.33	136.21203	352.36456	83812.908	42082.636
fabB	95.34278	123.58783	67.09773	43781.613	83831.382	3731.843	427.21287	634.53901	219.88672	293563.59	586611.92	515.26142	512.95259	62673.164	31593.058
fabD	15.95555	21.10149	10.80961	77696.413	121895.75	33497.079	258.45737	343.36174	173.553	55092.799	109873.91	311.69042	233.03536	27724.305	13978.67
fabF	37.763935	44.82802	30.69985	104417.64	197330.15	11505.13	411.112	504.57115	317.65285	279483.5	558792.88	174.11001	1012.7016	150945.93	75979.318
fabG	24.10711	23.33933	24.87489	31742.791	41057.882	22427.701	220.35078	162.81252	277.88904	17843.55	35264.229	422.87028	612.38422	34511.403	17561.893
fabH	28.486775	43.48839	13.48516	99381.002	167045.09	31716.912	330.62279	455.58608	205.65949	77685.366	155002.48	368.24776	408.29808	61022.411	30715.355
fabI	4.75549	4.98005	4.53093	140.4132	236.13215	44.69424	20.57086	27.64567	13.49605	1260.0911	2492.6234	27.55873	46.5709	1954.9614	1000.7662
fabZ	24.890935	27.04936	22.73251	7596.91	7193.8199	8000	168.3529	227.51753	109.18827	13424.148	26744.228	104.06898	198.24245	58603.92	29401.081
cydX	111.00395	142.99655	79.01134	1298.0944	365.60936	2230.5795	26.30256	21.2478	31.35732	910.00231	1743.8004	76.20426	30.43703	3163.123	1596.78

3H	3H	3H	pvals_0H	pvals_0.25 H	pvals_0.5H	pvals_1H	pvals_2H	pvals_3H
0.644905	0.7	0.58981	5.73E-04	7.91E-03	8.47E-04	1.98E-03	2.25E-02	7.93E-04
58125.457	116249.76	1.15683	1.28E-03	6.58E-03	5.65E-03	6.15E-05	2.25E-02	2.25E-02
3831.4076	7662.0492	0.76594	5.20E-03	2.06E-03	4.72E-05	1.62E-04	2.25E-02	2.25E-02
647.46134	1294.2696	0.65306	1.79E-02	1.35E-02	2.22E-03	3.13E-03	2.25E-02	2.26E-02
1548.5733	3096.0755	1.07116	3.01E-02	7.59E-03	3.86E-03	1.88E-02	2.25E-02	2.25E-02
829.20752	1657.9266	0.48841	2.31E-03	1.10E-02	7.82E-04	1.11E-03	2.25E-02	2.26E-02
91.33542	181.90287	0.76797	1.66E-02	1.78E-02	1.14E-03	1.72E-02	2.25E-02	2.26E-02
16700.509	33399.429	1.58843	8.61E-03	1.37E-03	1.81E-02	1.80E-02	2.25E-02	2.25E-02
6892.7985	13782.965	2.63205	1.18E-02	1.33E-02	1.15E-03	5.60E-05	2.25E-02	2.25E-02
0.862045	1.50689	0.2172	1.89E-03	5.08E-04	8.19E-04	3.48E-04	2.25E-02	7.47E-02
105298.83	210597.13	0.53444	3.94E-03	9.64E-03	4.66E-05	1.52E-08	2.25E-02	2.25E-02
0.00233	0.00356	0.00466	1.09E-03	4.99E-04	2.82E-04	2.87E-06	2.24E-02	1.82E-07
110861.24	221720.96	1.51701	7.31E-03	1.50E-02	5.62E-03	1.53E-02	2.25E-02	2.25E-02
126.56341	253.06694	0.05987	1.18E-02	1.42E-02	6.95E-04	7.54E-03	2.25E-02	2.28E-02
500.32099	999.5737	1.06828	1.23E-02	1.24E-02	1.69E-04	3.13E-06	2.25E-02	2.25E-02
1.86796	3.64786	0.08806	2.13E-02	1.14E-02	6.52E-02	2.31E-05	2.25E-02	4.87E-02
243.51476	485.92931	1.1002	8.01E-03	1.72E-02	7.28E-05	4.54E-04	2.25E-02	2.25E-02
5.701895	11.22698	0.17681	1.69E-03	7.70E-03	6.34E-03	2.83E-04	2.25E-02	2.78E-02
10055.249	20109.726	0.77185	7.54E-03	9.51E-03	1.56E-03	3.80E-03	2.25E-02	2.25E-02
2087967.1	4175933.4	0.85092	7.26E-03	1.56E-02	1.02E-02	1.72E-02	2.25E-02	2.25E-02
148029.97	296058.04	1.90452	3.93E-04	9.17E-03	9.26E-04	2.58E-02	2.25E-02	2.25E-02
309.73035	619.36109	0.09961	1.89E-03	1.74E-03	2.42E-04	5.76E-03	2.25E-02	2.26E-02
4.155815	8.14302	0.16861	1.17E-03	4.65E-02	6.21E-04	1.19E-03	2.25E-02	3.04E-02
212678.22	425351.88	4.57198	2.24E-04	1.92E-02	9.15E-03	1.98E-02	2.25E-02	2.25E-02
9707.5345	19414.995	0.07391	5.59E-05	1.69E-02	1.22E-02	1.40E-04	2.25E-02	2.25E-02
0.434315	0.46863	0.4	3.18E-04	1.84E-02	2.75E-03	2.24E-02	2.25E-02	1.22E-04
29.204865	58.39912	0.01061	1.57E-03	5.42E-05	5.03E-02	1.77E-03	2.25E-02	2.36E-02
31.829795	63.34087	0.31872	8.43E-04	5.24E-03	2.69E-03	6.22E-03	2.25E-02	2.32E-02
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
98.541245	196.02257	1.05992	2.40E-04	9.38E-03	3.90E-03	2.82E-02	2.25E-02	2.25E-02
77628.345	63459.108	91797.581	1.09E-02	5.06E-07	6.38E-05	1.52E-08	1.00E-04	1.09E-03
21.196285	0.02262	42.36995	1.10E-03	6.81E-06	3.90E-04	6.95E-07	1.11E-03	2.40E-02
0.00095	0.00187	0.00003	6.03E-02	3.04E-03	1.02E-02	5.18E-07	9.86E-02	2.83E-08
1.39358	0.13683	2.65033	2.79E-02	2.26E-04	3.19E-03	2.39E-06	1.93E-03	6.42E-02
255.47057	1.60978	509.33136	1.43E-02	4.30E-07	7.18E-03	3.71E-02	2.20E-02	2.25E-02
58.11854	0.22761	116.00947	6.76E-02	7.65E-04	1.54E-06	7.12E-08	6.11E-02	2.30E-02
1547.4709	26.80242	3068.1394	1.37E-02	5.16E-05	1.56E-02	1.93E-02	1.03E-02	2.20E-02
306.37218	1.50454	611.23981	1.60E-02	1.18E-02	3.57E-03	5.70E-02	2.05E-02	2.25E-02
46.726275	0.04614	93.40641	1.13E-02	8.73E-05	1.86E-03	3.02E-08	2.52E-03	2.32E-02
175.01311	0.00005	350.02616	1.68E-03	2.04E-03	9.68E-03	4.96E-07	3.60E-03	2.27E-02

176.42849	54.00019	298.85678	1.10E-03	3.94E-03	2.10E-03	2.15E-02	1.85E-02	1.31E-02
29.76715	0.28337	59.25093	5.83E-02	1.63E-04	1.92E-04	3.24E-08	1.16E-03	2.33E-02
75.886755	66.16221	85.6113	9.12E-04	1.34E-04	1.15E-02	3.71E-02	1.91E-02	5.57E-04
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
155548.58	311053.98	43.17622	1.71E-03	1.03E-02	1.60E-05	2.23E-02	2.16E-02	2.25E-02
13548.683	17494.631	9602.734	1.15E-02	1.42E-02	3.18E-03	2.25E-02	2.20E-02	2.71E-03
2193.092	262.31816	4123.8658	1.27E-03	1.56E-05	3.48E-04	2.16E-02	1.62E-02	1.88E-02
9525.9719	18158.248	893.69593	1.88E-03	1.30E-02	5.67E-04	2.22E-02	1.74E-02	1.96E-02
34517.116	66172.044	2862.1876	7.52E-03	1.95E-02	2.19E-02	2.25E-02	2.23E-02	2.00E-02
22842.983	34529.064	11156.902	2.86E-03	1.99E-02	7.06E-03	2.25E-02	2.20E-02	7.73E-03
14688.096	26275.758	3100.4342	3.73E-03	9.31E-03	3.44E-03	2.24E-02	2.20E-02	1.59E-02
5058994.6	10117947	41.68789	1.21E-03	1.91E-02	1.69E-03	2.25E-02	2.21E-02	2.25E-02
72418.804	144829.15	8.46126	3.68E-05	2.75E-03	2.22E-03	2.18E-02	2.15E-02	2.25E-02
48430.465	96860.93	0.0003	8.66E-03	1.26E-02	4.47E-03	2.24E-02	2.21E-02	2.25E-02
71192.781	135728.96	6656.599	1.19E-04	1.28E-02	4.09E-03	2.19E-02	2.11E-02	1.96E-02
14633.818	27267.636	2000	2.71E-04	9.37E-05	3.92E-03	2.23E-02	2.23E-02	1.83E-02
280.00028	557.59119	2.40937	2.71E-03	1.38E-02	1.30E-03	2.00E-02	2.20E-02	2.24E-02

Relative Gene expression JW15110 versus BW25113

	0H	0H	0H	0.25H	0.25H	0.25H	0.5H	0.5H	0.5H	1H	1H	1H	2H
folE	0.14632	0.90884	0.1224	0.00494	0.36326	1.9258	7.68537	0.56843	0.19936	1.0016	1.22297	0.53169	0.99543
nudB	0.05765	1.96608	0.09902	0.00021	0.62786	2.19147	0.28923	0.18772	0.066	1.00118	0.44726	0.10692	0.99518
folB	0.20386	0.87335	0.56355	0.0241	0.55121	2.37991	0.59231	0.59695	0.40422	1.00093	1.60455	0.7562	0.99883
folK	0.28353	0.54758	0.69529	0.0141	0.14268	3.13777	0.07191	0.15901	0.05357	0.99982	0.62906	0.33234	0.99902
folP	1.78267	0.72024	0.71763	0.03757	0.11668	0.60566	0.08037	0.19067	0.17958	1.00001	1.06053	0.31489	1.00053
aroF	0.24155	0.97845	0.08434	0.00131	0.07399	1.72439	0.24766	0.11636	0.05048	1.0002	0.21678	0.07226	0.99941
aroH	0.01519	0.41458	0.53553	1.76182	0.20052	1.76182	0.22772	0.34676	0.2542	1.00052	0.64733	0.20437	0.99831
aroG	0.3506	1.17782	0.07254	1.76182	0.13289	1.59301	0.36571	0.31579	0.09169	0.99916	1.05576	0.15287	0.99955
aroB	0.36948	1.27783	0.24314	0.00614	0.14006	1.79974	0.07759	0.28175	0.25357	1.00104	1.13994	0.50119	0.99935
aroD	1.85727	0.8489	0.91902	0.40386	0.64361	0.25173	0.77602	0.65246	0.08542	0.99981	0.9024	0.19326	0.99762
aroE	0.27344	0.76625	0.09328	0.0039	0.16402	0.74232	0.27242	0.27218	0.01665	1.00085	0.96009	0.28603	0.99728
ydiB	1.288	0.77947	1.25441	5.04717	1.967	0.05623	0.21645	1.21978	0.02898	0.99898	1.82805	0.10213	0.99359
aroK (aroL)	0.20914	0.83175	0.06948	0.00071	0.15851	1.91415	0.04283	0.35483	0.01066	1.00172	0.91399	0.41149	0.99657
aroA	0.85801	0.79911	0.26778	0.1027	0.32639	1.68728	0.2695	0.53198	0.11172	1.00046	1.14667	0.12691	0.99538
aroC	0.1009	0.61973	0.53468	0.00846	0.33945	1.82559	0.06391	0.34701	0.47882	1.00066	0.79921	0.06246	0.99793
pabBpabA	0.50701	0.62702	0.63307	0.0479	0.24261	0.88841	0.28482	0.43448	0.00467	0.9998	0.45925	0.27786	0.99688
pabC	0.09465	0.58145	0.28317	0.00444	0.15282	1.52738	6.21128	0.31787	0.01799	1.00054	0.77685	0.23543	0.99918
folC	0.13567	0.77883	0.7969	0.02309	0.14945	1.28931	0.28811	0.34431	0.16041	1.00067	0.99725	1.23855	0.99449
folA (folM)	0.17114	0.80316	0.35375	0.02292	0.45156	2.72397	0.84059	0.38153	0.48853	1.00108	1.75606	0.39368	0.99891
glyA	0.2035	1.17909	0.09657	0.02159	0.6372	5.17866	0.08589	0.35799	0.4903	1.00087	1.24387	0.23691	0.99939
gcvH	2.277	1.26065	0.63632	1.66384	0.60448	1.50771	1.26345	1.63595	3.03325	0.99902	3.8235	0.10806	1.00059
gcvP	2.2144	2.12724	0.05004	1.7819	0.77474	1.36355	1.79927	2.01926	1.88223	0.99956	5.41922	0.27685	1.00037
gcvT	1.63	1.18217	0.78088	0.61123	0.41487	0.83574	2.99578	0.72149	1.67908	0.99923	1.76056	0.50509	0.99531
lpdA	0.00201	2.81391	0.06992	0.00001	0.2944	5.48435	0.12764	0.36708	0.18405	1.00175	0.76857	2.32597	0.99855
folD	0.06341	0.74131	0.10606	0.00163	0.2232	3.55849	0.49638	0.14529	0.37402	1.00078	0.39871	0.22032	0.99902
ygfA (fau)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
metF	0.24979	1.16021	0.90677	0.00312	0.16895	0.36441	0.80945	0.26602	0.36817	0.7	0.64717	1.7142	1.00125
metH	1.08216	0.90153	0.66949	0.06268	0.51436	0.3097	0.99287	0.6907	0.49779	0.9991	1.02466	0.1986	1.00101
codB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
putP	0.19664	0.89072	0.84888	0.04234	0.14429	1.53935	2.25068	0.37096	1.26028	1.00047	1.44371	3.80635	0.99916
gadC	3.128	3.22655	0.07321	10	9.03392	0.02985	1.00015	6.24556	1.84242	0.99903	1.33903	0.59804	9.3352
dsdX	0.00017	1.04792	2.0446	11.70165	7.53659	0.03806	0.9998	2.57479	1.64486	0.99938	1.86484	0.10342	17.20828
adeQ	0.04036	1.53758	1.68375	15	15.96469	0.02287	1.00025	2.80437	1.95705	0.9999	3.3114	1.73312	5.69441
pnuC	0.05744	0.9218	0.46861	9.42092	0.89929	0.34928	1.00084	1.42067	4.33224	0.99933	0.56045	16.81245	0.00155
nupC	0.06399	0.88498	0.82266	3.27273	2.31798	1.43903	1.00003	1.24636	11.06143	1.0003	0.875	0.28726	7.52772
nupG	0.00674	1.01031	1.3884	9.69545	0.7924	0.11968	1.00026	1.95349	14.66983	0.99931	1.30372	1.30372	3.12268
panF	0.04409	1.38677	0.99636	0.10948	0.24142	0.18018	0.99984	0.55511	26.86518	1.00017	0.76469	1.05381	4.51549
aroP	0.13563	1.68538	1.26726	0.52426	1.28474	0.56857	1.00017	1.04218	4.82021	0.99991	0.40678	2.1284	23.60469
glcA	1.351	0.72718	1.56657	10	1.28288	0.01441	1.00041	1.75049	1.29854	0.99944	0.56352	5.36848	50.0055
uhpT	2.6273	1.22438	1.92491	5.59976	1.13242	0.06831	1.00007	1.33932	1.79802	0.99933	0.94041	1.07217	54.72637

cycA	1.9213	0.51445	0.5499	0.53099	0.36377	0.13826	0.99989	0.83374	4.64	1.00028	0.23059	1.57146	10.51957
citT	2.081	1.16871	1.72283	5	4.08565	0.02432	1.00002	1.78453	2.75561	0.99927	0.73989	0.33427	23.84274
shiA	1.11552	0.52138	0.39259	4.57836	0.99946	0.78259	1.00075	1.66036	7.1843	0.99985	0.2664	0.79694	6.91846
cysG	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
hcaT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
idnT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
accA	0.01576	0.68039	0.17965	0.02123	0.54929	4.85808	0.99931	0.60008	4.5988	1.00105	0.30021	1.14098	0.65937
accB	0.03451	0.2236	0.12364	0.00997	0.13531	0.45743	0.99974	0.70142	6.84054	1.00114	0.14231	2.10903	0.09154
accC	0.567	0.55043	0.60361	0.07793	0.19694	0.4676	1.00026	0.99389	6.05235	1.00043	0.13821	2.37588	1.69356
accD	0.1217	0.78125	0.28224	0.06758	1.41635	4.97299	0.99963	1.08743	7.32889	1.00135	0.92722	4.28456	1.15658
fabA	0.03711	1.03788	0.13834	0.00973	0.55278	12.76088	1.00037	0.86519	258.02484	1.00137	0.08606	1.16439	0.09533
fabB	0.48018	0.64837	0.07634	0.10492	0.37682	2.29279	0.99936	0.57606	5.73602	1.00122	0.2491	0.62894	0.48786
fabD	0.09721	2.00112	0.20625	0.02296	0.49174	12.56957	0.99951	1.30066	17.03982	1.00111	0.46541	2.26626	0.07615
fabF	0.03036	0.95797	0.11564	0.00163	0.83471	5.51029	0.99983	1.12083	8.20285	1.0013	0.1098	1.96576	0.69409
fabG	0.05664	1.25197	0.10763	0.01696	0.54273	4.82859	1.0002	1.61445	10.18525	1.0016	0.48638	1.45574	0.17101
fabH	0.05258	1.29782	0.17304	0.01477	0.25406	3.27314	0.99934	45	23.88507	1.00142	0.06521	2.42419	0.19576
fabI	0.02587	0.63883	0.28702	0.01208	0.5006	0.5006	1.00041	0.54928	17.67543	1.00147	0.06207	1.03673	0.00561
fabZ	0.05237	1.27282	0.11549	0.0053	0.45386	0	0.99997	0.59627	7.38775	1.00131	0.23314	1.97624	0.02
cydX	1.31	0.89221	0.06672	1.53504	18.59683	3.31356	1.00081	1.9535	7.29511	1.00158	2.82258	0.79488	2.1329

2H	2H	3H	3H	3H	pvals_0H	pvals_0.25H	pvals_0.5H	pvals_1H	pvals_2H	pvals_3H
0.01058	1.00462	0.00002	0.000246	0.000133	1.43E-02	7.28E-02	5.33E-02	7.29E-02	4.23E-02	4.26E-10
0.01088	0.99941	0.00029	0.003567	0.0019285	6.88E-02	9.35E-02	6.15E-04	2.06E-02	4.19E-02	8.98E-08
0.10938	0.9994	0.0117	0.14391	0.077805	1.44E-02	9.85E-02	1.78E-03	6.80E-02	4.22E-02	1.71E-04
0.02316	0.99905	0.00078	0.009594	0.005187	5.52E-03	9.32E-02	1.29E-04	2.15E-02	4.22E-02	6.54E-07
0.05565	0.99888	0.00039	0.004797	0.0025935	8.55E-02	5.22E-03	1.70E-04	4.76E-02	4.22E-02	1.63E-07
0.03813	0.99961	0.00018	0.002214	0.001197	1.77E-02	5.51E-02	4.49E-04	1.86E-02	4.22E-02	3.46E-08
0.02151	0.99839	0.00148	0.018204	0.009842	4.98E-03	6.88E-02	2.48E-04	2.39E-02	4.21E-02	2.38E-06
0.00203	0.99809	0.00009	0.001107	0.0005985	2.95E-02	7.83E-02	1.26E-03	4.61E-02	4.21E-02	8.63E-09
0.18859	0.99985	0.04613	0.567399	0.3067645	3.74E-02	6.04E-02	6.38E-04	6.01E-02	4.22E-02	4.40E-03
0.00931	0.99903	0.0128	0.15744	0.08512	5.87E-02	3.82E-03	1.45E-02	3.57E-02	4.21E-02	2.08E-04
0.02695	1.00351	0.00009	0.001107	0.0005985	9.05E-03	8.99E-03	1.08E-03	3.92E-02	4.23E-02	8.63E-09
0.00773	1.00728	0.003	0.0369	0.01995	5.81E-02	4.49E-02	3.01E-02	9.67E-02	4.23E-02	9.97E-06
0.18493	1.00318	0.0305	0.37515	0.202825	1.15E-02	6.64E-02	1.58E-03	3.47E-02	4.22E-02	1.52E-03
0.04444	0.99897	0.00018	0.002214	0.001197	1.96E-02	6.12E-02	2.97E-03	5.27E-02	4.19E-02	3.46E-08
0.0575	1.00093	0.00248	0.030504	0.016492	6.86E-03	6.71E-02	2.90E-03	3.15E-02	4.22E-02	6.77E-06
0.00855	0.99829	0.00013	0.001599	0.0008645	9.83E-04	1.39E-02	2.65E-03	1.92E-02	4.20E-02	1.80E-08
0.0165	0.99955	0.00388	0.047724	0.025802	4.08E-03	4.61E-02	6.17E-02	2.84E-02	4.22E-02	1.69E-05
0.05596	1.00398	0.00314	0.038622	0.020881	1.87E-02	3.31E-02	5.42E-04	4.28E-02	4.22E-02	1.09E-05
0.23367	0.99796	0.04816	0.592368	0.320264	9.73E-03	9.44E-02	9.02E-03	9.10E-02	4.20E-02	4.95E-03
0.05236	0.99867	0.00197	0.024231	0.0131005	2.79E-02	6.20E-02	2.86E-03	6.26E-02	4.21E-02	4.24E-06
0.01233	0.99802	0.00056	0.006888	0.003724	4.99E-02	5.15E-02	2.11E-02	6.24E-02	4.22E-02	3.36E-07
0.01984	0.99909	0.00071	0.008733	0.0047215	5.79E-02	4.04E-02	5.04E-04	5.24E-02	4.22E-02	5.42E-07
0.01015	0.99903	0.00029	0.003567	0.0019285	5.05E-02	8.92E-03	3.49E-02	8.31E-02	4.19E-02	8.98E-08
0.08632	0.99957	0.00461	0.056703	0.0306565	9.71E-02	6.55E-02	8.61E-04	5.30E-02	4.21E-02	2.41E-05
0.05601	0.99843	0.0492	0.60516	0.32718	8.64E-03	8.42E-02	2.34E-03	1.91E-02	4.21E-02	5.25E-03
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.01933	0.99998	0.01385	0.170355	0.0921025	4.90E-02	1.58E-03	8.96E-03	9.58E-02	4.23E-02	2.47E-04
0.01387	0.99933	0.0007	0.00861	0.004655	4.35E-02	3.27E-03	1.99E-02	4.40E-02	4.23E-02	5.26E-07
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.02417	0.99885	0.00781	0.096063	0.0519365	2.55E-02	4.72E-02	6.42E-02	3.39E-02	4.21E-02	7.21E-05
0.08074	0.03095	0.00195	0.023985	0.0129675	3.85E-02	2.34E-02	3.38E-02	9.30E-02	5.59E-02	4.15E-06
0.03921	1.05524	0.08753	1.076619	0.5820745	9.63E-02	2.53E-02	2.47E-02	9.85E-02	4.56E-02	2.81E-02
0.08048	15.41122	0.01594	0.196062	0.106001	8.83E-02	2.12E-02	2.19E-02	2.75E-02	3.09E-02	3.37E-04
0.04597	0.05	0.00513	0.063099	0.0341145	1.74E-02	4.76E-02	3.55E-02	4.39E-02	2.57E-05	3.00E-05
0.10572	0.23659	1.64535	18.42792	10.036635	2.61E-02	1.27E-02	4.09E-02	3.32E-02	5.76E-02	2.03E-02
0.08397	4.42467	0.45997	5.657631	3.0588005	6.78E-02	4.98E-02	3.84E-02	1.84E-02	3.53E-02	3.04E-02
0.13032	0.92683	0.79335	9.758205	5.2757775	6.79E-02	2.14E-04	4.33E-02	5.66E-02	5.90E-02	2.40E-02
0.15652	0.357	0.55935	6.880005	3.7196775	9.55E-02	4.88E-02	4.16E-02	7.58E-02	4.61E-02	2.75E-02
0.04292	0.0977	0.17445	2.145735	1.1600925	4.83E-02	4.71E-02	2.50E-02	4.83E-02	4.45E-02	8.05E-02
0.01394	0.4518	0.37573	4.621479	2.4986045	1.50E-02	5.33E-02	2.43E-02	9.27E-02	4.39E-02	3.46E-02

0.07748	1.12861	0.96991	11.929893	6.4499015	9.93E-02	2.88E-03	4.50E-02	8.81E-02	4.73E-02	2.27E-02
0.04685	0.03475	0.15981	1.965663	1.0627365	1.32E-02	3.14E-02	2.37E-02	2.52E-02	4.72E-02	9.15E-02
0.04416	0.10106	0.05207	0.640461	0.3462655	2.83E-02	4.59E-02	3.65E-02	2.89E-02	6.13E-02	6.14E-03
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.126	0.27547	0.09705	1.193715	0.6453825	7.13E-03	6.50E-02	4.90E-02	5.49E-02	5.54E-03	3.79E-02
0.10335	33.744	0.15255	1.876365	1.0144575	3.89E-04	2.67E-03	4.53E-02	8.96E-02	4.55E-02	9.79E-02
0.05672	0.43305	0.50175	6.171525	3.3366375	1.36E-04	2.27E-03	4.23E-02	8.17E-02	6.38E-02	2.90E-02
0.11242	0.27551	0.19854	2.442042	1.320291	9.30E-03	5.13E-02	4.15E-02	4.35E-02	2.73E-02	6.70E-02
0.10226	2.87841	0.47496	5.842008	3.158484	2.02E-02	4.95E-02	4.23E-02	5.35E-02	9.81E-02	2.98E-02
0.09391	0.27397	0.39406	4.846938	2.620499	7.19E-03	9.23E-02	4.77E-02	2.27E-02	2.45E-03	3.35E-02
0.08554	1.90755	0.1677	2.06271	1.115205	7.43E-02	4.99E-02	4.12E-02	6.92E-02	6.61E-02	8.53E-02
0.07336	0.23611	0.23145	2.846835	1.5391425	1.66E-02	5.82E-02	4.13E-02	9.66E-02	6.99E-03	5.49E-02
0.05689	0.72235	0.42557	5.234511	2.8300405	3.09E-02	6.53E-02	3.85E-02	9.53E-02	7.98E-03	3.18E-02
0.0548	1.52658	0.38494	4.734762	2.559851	3.40E-02	8.79E-02	2.21E-02	8.34E-02	4.76E-02	3.40E-02
0.16405	0.25401	0.015	0.1845	0.09975	6.15E-03	5.55E-03	4.38E-02	4.47E-02	7.07E-04	2.94E-04
0.02012	0.02	1.33806	16.458138	8.898099	3.20E-02	3.01E-03	4.60E-02	9.02E-02	1.67E-10	2.12E-02
0.05605	1.48165	0.17794	2.188662	1.183301	5.73E-02	3.35E-02	3.43E-02	4.90E-02	7.50E-02	7.82E-02

Relative gene expression of BW25113 reference strain treated with Trimethoprim

	TM0_1	TM0_2	TM0_3	TM0_4	TM0_5	TM0_6	TM1_1	TM1_2	TM1_3	TM1_4	TM1_5	TM1_6	TM2_1	TM2_2	TM2_3
folE	0.36526	0.78575	0.18864	0.57972	8.33435	12.44627	0.07828	0.03171	10.22857	3.44574	N/A	N/A	16.6308	4.89199	0.67712
nudB	0.59139	0.48655	0.79922	0.25515	2.76082	7.62449	25.7612	1.96097	0.37315	0.07304	N/A	N/A	27.04895	0.10799	12.79038
folB	0.67976	1.46232	1.00293	3.08208	0.64068	0.95678	0.39662	0.16069	8.806	2.96651	26.76261	7.0286	1.92057	0.56494	294.12125
folK	1.32881	1.09325	0.6752	0.21556	1.40806	3.88862	2.10922	0.16056	0.26505	0.05188	N/A	N/A	2345.2761	9.36294	3.03182
folP	0.39862	0.85753	0.53284	1.63745	0.51983	0.7763	1.03646	0.4199	2.21088	0.74479	35.60461	9.35075	1.11291	0.32736	35.53351
aroF	1.81661	1.49458	2.37167	0.75716	5.61169	15.49767	0.81689	0.06218	12.5303	2.4528	N/A	N/A	22.95957	0.09166	3.11704
aroH	0.46404	0.99826	0.53905	1.65655	0.26344	0.39342	2.06612	0.83706	1.58474	0.53386	38.77918	10.18448	2.86112	0.84161	5.00824
aroG	0.16213	0.13339	7.41593	2.36755	1.21426	3.35339	3.15176	0.23992	4.91502	0.96211	0.00059	0.00059	0.35215	0.00141	1.61172
aroB	0.17732	0.38147	1.35156	4.15345	0.71999	1.07521	1.01708	0.41206	1.22168	0.41155	179.56178	47.15788	2.67598	0.78715	142.88444
aroD	1.64903	1.3567	652.27863	208.24152	0.10937	0.30205	0.41973	0.03195	729.1358	142.72822	N/A	N/A	6.57478	0.02625	6.25491
aroE	0.66474	1.43	0.51443	1.58087	1.42212	2.12375	0.21466	0.08697	10.15	3.41927	12.81845	3.36648	0.25152	0.07399	12.68532
ydiB	0.59528	0.48975	2.06643	0.65971	0.11116	0.307	0.70086	0.05335	1.38586	0.27128	168.8177	168.8177	218.21572	0.87117	0.10925
aroK(aroL)	0.1472	0.31666	0.23735	0.72939	1.62355	2.42456	0.16354	0.06626	2.73661	0.92189	N/A	N/A	4.28697	1.26102	2.06241
aroA	0.54688	0.44993	0.37094	0.11842	2.17291	6.00089	0.60608	0.04614	3.20495	0.62737	34.93191	34.93191	6.54396	0.02613	10.97559
aroC	0.7041	1.51467	0.84144	2.58581	0.29526	0.44094	0.29901	0.12114	0.46185	0.15558	8.70937	2.28732	0.9574	0.28162	0.57282
pabBpabA	7.62834	6.27603	0.31803	0.10153	0.04838	0.1336	4.43167	0.33734	1946.9088	381.1071	N/A	N/A	119.44814	0.47687	0.03827
pabC	0.61429	1.32147	0.33335	1.0244	1.01058	1.50918	1.72865	0.70034	4.22898	1.42463	97.34178	25.56464	3.03626	0.89312	34.56321
folC	1.36309	1.12145	2.03681	0.65026	0.37478	1.03501	0.88799	0.06759	23.18419	4.5383	N/A	N/A	1137.6157	4.54165	3.04928
folA(folM)	0.24651	0.53029	0.16351	0.50247	0.71221	1.0636	0.31014	0.12565	58.50645	19.70929	4.14723	1.08918	5.00979	1.47364	1.25231
glyA	0.9131	0.75123	0.77609	0.24777	0.6838	1.88845	1.32403	0.10079	0.30287	0.05929	13.37666	13.37666	24.91178	0.09945	13.53528
gcvH	3.17323	6.82632	0.53349	1.63946	0.79614	1.18892	0.19002	0.07698	0.14633	0.0493	0.44373	0.11654	0.00364	0.00107	1.03528
gcvP	8.83041	7.26501	1.76408	0.56319	1.21912	3.36682	2.90056	0.22079	0.0346	0.00677	4.05252	4.05252	0.0724	0.00029	0.61464
gcvT	3.07319	6.61112	0.29328	0.90127	0.54496	0.81382	0.0147	0.00595	0.37775	0.12725	N/A	N/A	0.21338	0.06277	0.00017
lpdA	1.24557	1.02476	0.90305	0.2883	7.99139	22.06963	1.62462	0.12367	20.24045	3.96207	0.07944	0.07944	13.23221	0.05283	11.93001
folD	0.44959	0.96716	1.34489	4.13295	1.49373	2.23068	0.77458	0.31381	1.94945	0.65672	24.65737	6.4757	2.59685	0.76387	53.27228
ygfA(fau)	3.24905	2.67308	0.06971	0.02226	306.66576	846.91179	0.2401	0.01828	169043.53	33090.246	N/A	N/A	12.39328	0.04948	445.6963
metF	0.50174	1.07936	1.26067	3.87412	0.59521	0.88886	2.54641	1.03164	1.74772	0.58876	8.03094	2.10915	3.14149	0.92408	26.98028
metH	2.09631	1.72469	3.01165	0.96148	1.07655	2.97307	11.03219	0.83978	N/A	N/A	37147.332	37147.332	4.68673	0.01871	2.92835
codB	0.78602	1.6909	1.76015	5.40906	0.46143	0.68908	1.41337	0.57261	9.23092	3.10965	4.95275	1.30073	6.00246	1.76564	0.19666
putP	5.82989	4.7964	1.12191	0.35817	N/A	N/A	1.7512	0.1333	0.10828	0.02119	438.22448	438.22448	0.46988	0.00188	1.44841
gadC	0.73873	1.58916	1.69004	5.19361	0.80303	1.19922	0.9765	0.39562	1.48575	0.50051	2.51654	0.66091	0.00144	0.00042	0.05443
dsdX	3.63345	2.98933	1.67	0.53315	0.24091	0.66531	8.0756	0.61472	0.72059	0.14106	N/A	N/A	2.01798	0.00806	2.49776
adeQ	0.60233	1.29574	1.7467	5.36774	0.47046	0.70257	1.25254	0.50745	133.92173	45.11473	468.55468	123.05538	10.85048	3.19169	N/A
pnuC	0.53061	0.43654	0.58055	0.18534	0.19134	0.52842	1.57818	0.12013	2.30457	0.45112	1.1135	0.99733	32.74395	0.13072	400.74948
nupC	1.17304	2.52347	0.75414	2.31753	0.40859	0.61017	1.5603	0.63213	1.74759	0.58872	57.46549	15.09202	2.96244	0.87141	6.80025
nupG	1.3766	1.13256	1.81846	0.58055	0.15568	0.42995	0.70904	0.05397	0.06524	0.01277	N/A	N/A	17.36109	0.06931	184.65869
panF	0.46485	N/A	0.32541	N/A	0.66963	N/A	2.46831	N/A	2.96847	N/A	3.80767	N/A	3.3996	N/A	45.14203
aroP	1.716	1.4118	0.96005	0.3065	1.09265	3.01755	9.32967	0.71019	0.25152	0.04924	739.93365	739.93365	0.8439	0.00337	3.32443
glcA	0.7114	1.53038	0.25327	0.77832	0.63703	0.95133	7.26403	2.94291	1.6429	0.55345	2.74593	0.72116	1.6993	0.49985	4.38479

Relative gene expression of BW25113 reference strain treated with Trimethoprim

uhpT	1.71313	1.40944	12.50629	3.99266	1.29978	3.58957	7.23705	0.55089	0.62771	0.12287	7.29889	7.29889	28.80599	0.115	0.23953
cycA	1.57642	3.39123	0.17718	0.5445	0.74746	1.11623	1.19225	0.48302	22.44629	7.56157	42.02519	11.03698	0.25736	0.0757	7.78721
citT	2.47865	2.03925	18.67048	5.9606	1.38135	3.81485	187.29752	14.2573	0.2991	0.05855	2.62905	2.62905	5.81502	0.02322	2.07053
shiA	0.9985	2.14799	0.48208	1.48147	0.93963	1.40321	0.77064	0.31221	1.09822	0.36996	16.76865	4.40391	0.15579	0.04583	0.73925
cysG	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
hcaT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
idnT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
accA	0.33784	0.72677	0.3158	0.97048	2.21474	3.30743	1.04117	0.42182	2.03649	0.68604	141.20046	37.08313	1.95747	0.57579	26.49026
accB	0.57399	0.47224	0.60331	0.19261	0.64158	1.77183	5.75893	0.43838	0.12313	0.0241	90.56545	90.56545	37.64669	0.1503	8.73355
accC	0.17431	0.37498	0.33162	1.01909	0.68849	1.02818	1.59147	0.64476	0.86882	0.29268	5.29279	1.39003	0.99824	0.29363	11.07038
accD	0.22106	0.18187	0.6662	0.21269	1.10274	3.0454	2.67098	0.20332	0.12614	0.02469	3.44771	3.44771	24.41141	0.09746	0.94544
fabA	0.2291	0.49284	0.2142	0.65824	1.87667	2.80256	0.32394	0.13124	3.2923	1.10909	1.51517	0.39792	21.3928	6.29274	125.83787
fabB	0.22765	0.18729	13.06729	4.17176	6.74035	18.61467	20.75703	1.58005	0.10648	0.02084	0.86473	0.86473	186.96306	0.7464	26.34014
fabD	0.96763	2.08158	0.39096	1.20146	4.07336	6.08303	0.09018	0.03653	4.22548	1.42345	0.00824	0.00216	12.50615	3.67871	16.94406
fabF	0.33679	0.27709	2.88077	0.91969	11.23155	31.0179	2.78302	0.21185	0.2168	0.04244	3.78148	3.78148	19.18531	0.07659	64.71332
fabG	0.62056	1.33497	0.20232	0.62175	2.47491	3.69596	0.15682	0.06353	3550.3133	1196.0078	9.02642	2.37059	2.92978	0.8618	5.01389
fabH	0.39028	0.32109	0.39609	0.12645	5.61576	15.50893	2.00529	0.15264	0.21672	0.04242	0.03057	0.03057	119.9484	0.47886	75.30697
fabI	0.27854	0.5992	0.44703	1.37375	1.32071	1.97231	0.5463	0.22132	0.91683	0.30886	0.24832	0.06521	N/A	N/A	7.66479
fabZ	0.44569	0.36668	46.24944	14.76524	872.31669	2409.0569	0.00125	0.0001	29037.841	5684.153	N/A	N/A	4.18941	0.01673	N/A
cydX	1.66028	3.57163	11.1427	34.24234	3.64911	5.44948	0.07858	0.03184	0.4941	0.16645	7.67901	2.01672	0.34754	0.10223	1.98782

TM2_4	TM2_5	TM2_6	TM3_1	TM3_2	TM3_3	TM3_4	TM3_5	TM3_6	pvals_0H	pvals_1H	pvals_2H	pvals_3H
0.015	N/A	N/A	9311.6605	39.81951	48.49194	1.84281	10000.973	134.85068	2.53E-02	3.83E-02	3.22E-02	1.69E-02
12.79038	5.38E+009	5.38E+009	2.0267	20.6105	1.77865	1.77865	72.15259	72.15259	3.95E-02	4.05E-02	1.75E-02	1.10E-02
6.51546	0.45093	0.64525	N/A	N/A	33.48532	1.27252	6115.41	82.4587	4.55E-02	1.62E-02	3.54E-02	3.81E-02
3.03182	21.59205	21.59205	6.02083	61.22888	5.2993	5.2993	0.096	0.096	4.44E-02	5.23E-02	3.51E-02	2.71E-02
0.78715	0.77029	1.10224	22.45896	0.09604	6.52902	0.24812	180.32008	2.43139	2.99E-02	2.57E-02	3.77E-02	2.92E-02
3.11704	N/A	N/A	0.7828	7.96068	0.09789	0.09789	1.84817	1.84817	1.77E-02	3.82E-02	3.16E-02	4.04E-02
0.11094	65098.438	93151.927	N/A	N/A	0.07249	0.00275	446.39765	6.01912	2.45E-02	2.49E-02	1.83E-02	3.87E-02
1.61172	203.04013	203.04013	0.1163	1.18276	0.09249	0.09249	0.11338	0.11338	2.54E-02	5.41E-02	1.75E-02	1.05E-03
3.16522	5.43805	7.78152	N/A	N/A	8.88107	0.3375	352.99336	4.75968	6.25E-02	2.58E-02	3.11E-02	3.74E-02
6.25491	0.02733	0.02733	0.62161	6.32142	0.0694	0.0694	0.21807	0.21807	2.40E-02	3.00E-02	1.82E-02	8.13E-02
0.28101	12260785	17544441	N/A	N/A	0.28055	0.01066	6017.7052	81.14127	2.92E-02	1.22E-02	1.83E-02	3.84E-02
0.10925	16.15118	16.15118	0.24615	2.50318	0.00109	0.00109	0.18759	0.18759	3.47E-02	1.77E-02	3.00E-02	2.83E-02
0.04569	3.52312	5.04138	N/A	N/A	241.73434	9.1865	1299958.5	17528.323	8.26E-02	9.67E-02	8.04E-03	3.84E-02
10.97559	0.21005	0.21005	1.2375	12.58479	0.07035	0.07035	4650.6131	4650.6131	5.40E-02	1.72E-02	1.42E-02	1.74E-02
0.01269	23.33771	33.39486	14.76492	0.06314	2.98893	0.11359	0.20687	0.00279	8.63E-02	4.99E-02	2.06E-02	4.37E-02
0.03827	N/A	N/A	1.96973	20.03115	2.38815	2.38815	0.00042	0.00042	3.72E-02	2.98E-02	4.02E-02	3.21E-02
0.76565	1795.6965	2569.5331	193.54019	0.82764	2.55111	0.09695	3.82354	0.05156	8.68E-02	2.39E-02	1.79E-02	3.57E-02
3.04928	N/A	N/A	3.34368	34.00355	0.03319	0.03319	0.00004	0.00004	6.99E-02	3.38E-02	3.87E-02	3.91E-02
0.02774	7.14222	10.22009	388.31737	1.66056	250.05905	9.50286	0.58509	0.00789	1.77E-03	2.26E-02	1.07E-02	1.81E-02
13.53528	N/A	N/A	9.01565	91.68471	3.44765	3.44765	3.69751	3.69751	6.03E-02	2.28E-02	9.86E-03	2.67E-02
0.02293	35.99515	51.50688	N/A	N/A	0.56045	0.0213	2.94497	0.03971	2.20E-02	3.09E-06	2.02E-02	8.86E-02
0.61464	N/A	N/A	0.02344	0.23836	0.15833	0.15833	1.04954	1.04954	9.87E-03	3.33E-02	2.76E-03	3.49E-03
0	570.52185	816.38226	N/A	N/A	0.42645	0.01621	0.39882	0.00538	3.47E-02	2.11E-04	1.84E-02	6.54E-04
11.93001	0.00006	0.00006	1.01924	10.36515	0.05995	0.05995	754.97389	754.97389	2.47E-02	3.48E-02	1.20E-02	1.72E-02
1.1801	5.98563	8.56507	40.12801	0.1716	23.68333	0.90002	954.83874	12.87481	2.06E-02	2.71E-02	2.42E-02	3.25E-02
445.6963	51.63562	51.63562	35.59419	361.97518	36.95097	36.95097	0.14117	0.14117	2.28E-02	2.98E-02	1.17E-02	2.32E-02
0.59768	3.46795	4.96243	32.31552	0.13819	10.31285	0.39191	658.515	8.87926	5.08E-02	1.91E-02	2.26E-02	3.27E-02
2.92835	0.06586	0.06586	0.3133	3.18615	0.13073	0.13073	0.38116	0.38116	4.42E-03	1.82E-02	3.83E-02	6.36E-02
0.00436	0.00004	0.00006	146.91432	0.62825	1.20698	0.04587	0.0262	0.00035	3.38E-02	1.27E-02	7.50E-02	3.74E-02
1.44841	0.164	0.164	0.07486	0.76127	0.1636	0.1636	3.99426	3.99426	2.29E-02	1.76E-02	2.15E-02	5.34E-02
0.00121	0.00054	0.00078	0.00269	0.00001	0.13482	0.00512	0.20004	0.0027	2.60E-02	7.96E-02	1.13E-10	1.47E-07
2.49776	480.85436	480.85436	0.0885	0.90005	0.33436	0.33436	0.06235	0.06235	3.29E-02	5.18E-02	1.73E-02	3.09E-04
N/A	0	0	1552.1081	6.63729	177.39771	6.74155	4327.0052	58.34428	4.01E-02	1.36E-02	3.99E-02	2.07E-02
400.74948	0.15093	0.15093	1.19135	12.11545	0.44053	0.44053	434.05828	434.05828	4.44E-05	7.80E-02	1.57E-02	1.69E-02
0.15064	103.60105	148.24684	279.10585	1.19354	2.19677	0.08348	0.02257	0.0003	4.58E-02	2.55E-02	1.69E-02	3.66E-02
184.65869	N/A	N/A	0.57242	5.82121	36.42228	36.42228	21.39168	21.39168	7.57E-02	1.78E-03	1.57E-02	2.50E-03
N/A	0.69884	N/A	233.84671	N/A	26.31408	N/A	74.16331	N/A	3.59E-03	3.35E-03	3.96E-02	2.20E-02
3.32443	0.00105	0.00105	0.81385	8.27649	1.21214	1.21214	4.14463	4.14463	3.15E-02	1.72E-02	7.24E-02	1.06E-02
0.09713	0.28839	0.41267	104.97557	0.44891	9.77097	0.37132	14.35058	0.1935	3.21E-02	1.64E-02	7.46E-02	2.74E-02

Relative gene expression of BW25113 reference strain treated with Trimethoprim

0.23953	0.82262	0.82262	0.37248	3.78792	11.15552	11.15552	12.71306	12.71306	1.38E-02	1.21E-02	4.18E-02	1.60E-03
0.1725	0.61577	0.88112	67.03153	0.28665	34.39556	1.30712	384.05076	5.17845	6.05E-02	9.81E-03	6.31E-02	2.44E-02
2.07053	0.06515	0.06515	0.20227	2.05699	4.3761	4.3761	0.23905	0.23905	1.37E-02	3.24E-02	4.90E-02	3.21E-02
0.01638	0.02716	0.03887	47.93253	0.20497	1.58697	0.06031	N/A	0.01348	3.47E-02	3.14E-02	8.18E-05	3.99E-02
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	5.52E-02	2.56E-02	2.91E-02	3.54E-02
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.58682	2.36863	3.38936	938.26843	4.01232	16.24866	0.61749	0.12047	0.00162	N/A	N/A	N/A	N/A
8.73355	224.69149	224.69149	4.87964	49.62354	16.53597	16.53597	21.08012	21.08012	2.48E-02	1.68E-02	1.22E-02	1.97E-03
0.24523	2.65656	3.80137	674.75935	2.88548	20.95324	0.79627	2.88271	0.03887	4.51E-03	4.05E-02	2.51E-02	3.46E-02
0.94544	N/A	N/A	2.56381	26.07266	0.92865	0.92865	1.06228	1.06228	8.42E-02	3.91E-02	4.15E-02	3.32E-02
2.7876	138.64925	198.39869	14564.777	62.28344	10.34182	0.39301	0.01416	0.00019	9.20E-02	8.01E-02	6.16E-03	3.61E-02
26.34014	2.50498	2.50498	4.30491	43.77875	1.1081	1.1081	1.20772	1.20772	9.58E-03	4.07E-02	2.36E-02	3.18E-02
0.37535	73837325	105656746	6269.1386	26.80875	2.23371	0.08489	4843703.8	65311.323	1.62E-02	9.61E-02	1.83E-02	3.56E-02
64.71332	N/A	N/A	2.67789	27.23283	0.69152	0.69152	0.85405	0.85405	2.29E-02	3.34E-02	1.14E-02	3.49E-02
0.11107	1.71977	2.46089	1352.9869	5.78579	0.7739	0.02941	24648.098	332.34895	4.11E-02	2.34E-02	1.54E-02	3.29E-02
75.30697	435.45013	435.45013	7.23571	73.58356	0.56221	0.56221	0.00321	0.00321	3.27E-02	1.26E-02	6.21E-03	3.41E-02
0.16979	24007.098	34352.705	265.03585	1.13337	0.81254	0.03088	0.04082	0.00055	9.96E-02	4.26E-04	1.91E-02	3.69E-02
N/A	N/A	N/A	4.41348	44.88289	360231.24	360231.24	0.0053	0.0053	2.19E-02	2.98E-02	6.90E-02	1.75E-02
0.04403	0.04133	0.05914	2.22459	0.00951	1.33319	0.05066	0.02128	0.00029	1.36E-02	5.70E-02	1.30E-02	3.57E-02