Novel antibiotic mode of action by repression of promoter isomerisation

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Abstract:

Rising levels of antibiotic resistance dictate that new antibiotics with novel modes of action must be found. Here, we investigated the mode of action of a novel antibiotic that is a member of a family of synthetic DNA minor groove binding (MGB) molecules. MGB-BP-3 has successfully completed a Phase II clinical trial in humans as an orally administered drug for the treatment of chronic *Clostridioides* (*Clostridium) difficile* infections, where it outperformed the existing benchmark (vancomycin). MGB-BP-3 is active against a variety of Gram-positive pathogens including *Staphylococcus aureus,* which was used as the model for this study. The transcriptomic response of *S. aureus* to MGB-BP-3 identified downregulated promoters. DNase I and permanganate footprinting demonstrated binding to essential SigA promoters and the inhibition of promoter isomerisation by RNA polymerase holoenzyme. Promoters controlling DNA replication and peptidoglycan biosynthesis are amongst those affected by MGB-BP-3. Thus, MGB-BP-3 binds to and inhibits multiple essential promoters on the *S. aureus* chromosome, suggesting that evolution of resistance by drug target mutation should be unlikely. In confirmation, laboratory-directed evolution against sub-inhibitory concentrations of MGB-BP-3 resulted in no resistance whereas resistance to the single target RNA-polymerase inhibitor rifampicin arose rapidly.

Introduction:

DNA minor-groove binding (MGB) drugs have a variety of effects on infectious agents including bacteria, fungi, parasites and viruses [1]. Distamycin, a natural product made by *Streptomyces*, has been used as a chemical biology probe to examine the structure of AT-rich promoter UP elements in *Escherichia coli* and has shown promise as an anti-cancer agent [2, 3]. Distamycin inhibits the binding of the transcriptional

machinery to DNA [4, 5], but its toxicity to humans has prevented it from being developed as an anti-infective agent. Using distamycin as a design concept, a family of synthetic MGBs was synthesised [*e.g.* 6]. These structural variants retained DNA-binding and anti-Gram-positive activity but lacked human toxicity [6]. One of these, MGB-BP-3 (Figure 1a), has been taken forward for clinical development. It has strong (<1 μg/ml) antibacterial activity against methicillin-resistant and -susceptible *Staphylococcus* species, pathogenic *Streptococcus* species, vancomycin-resistant and susceptible *Enterococcus,* and *Clostridioides* (*Clostridium*) *difficile*. Its oral formulation, developed for the treatment of *C. difficile* infections, has successfully completed a Phase II clinical trial. Subsequently, different compounds have been found to be active against a wide variety of pathogens and some cancer cell lines [7-11]. Antibacterial activity of MGB-BP-3 is confined to Gram-positive bacteria and dose response curves show a steep decrease in viability over a narrow concentration range, suggesting a catastrophic failure in the bacterium rather than an interaction with a single receptor typified by a sigmoidal dose-response curve. Members of this MGB family have typically been shown to bind to 6–8 base pairs in dsDNA [6, 12]. Whilst it can be reasonably anticipated that the main mode of action of MGB-BP-3 is by binding to DNA, the detailed biological consequences of binding to DNA that eventually lead to cell death cannot be predicted and need further investigation. Moreover, a short minor groove binder such as MGB-BP-3 could bind to many sites on the bacterial genome with the potential to interfere with a number of essential biological processes. Here, we have used RNA-Sequencing to probe the transcriptional consequences of MGB-BP-3 binding to the *S. aureus* chromosome. This approach, along with qPCR melt analysis, phenotypic microarrays, DNase I footprinting and potassium

permanganate footprinting allows the identification of promoters that are sensitive to MGB-BP-3 shedding new light on its mode of action against *S. aureus*.

These data indicate multiple chromosomal binding sites, suggesting that generation of resistance to this type of drug would be significantly less than for drugs with a single target. In order to test this hypothesis we used a directed evolution approach to compare the evolvability of resistance to MGB-BP-3 and rifampicin.

Together, these results provide a comprehensive profile of the effect of MGB-BP-3 on *S. aureus* in culture and support the concept of multiple antibacterial actions via selective, but simultaneous, inhibition of a subset of promoters.

Materials and Methods:

Bacterial strain and growth conditions:

S. aureus subsp. *aureus* strain NCTC8325 from the HPA Culture Collection was used throughout this study with the exception of the resistance/evolution experiment for which *S. aureus* ATCC 43300 (resistant to methicillin and oxacillin) was used. Bacteria were grown for transcriptomic analyses according to the following: pre-cultures were prepared by inoculation from a frozen bead stock (Microbank, Fisher Scientific UK) to 5 ml of Tryptic Soya Broth (Sigma-Aldrich) and incubated overnight. Six 250 ml shake flasks with 50 ml of cation-adjusted Mueller-Hinton broth 2 (Sigma-Aldrich) were inoculated to OD_{600} of 0.05 using a pre-culture. All cultures were incubated at 250 rpm and 37°C. At OD_{600} of 0.3, 0.5 x MIC of MGB-BP-3 dissolved in DMSO was added to the cultures in triplicate (treated samples), whereas control samples (also in triplicate) where treated with same volume of DMSO only (untreated samples). Samples of 10 ml were withdrawn 10 min after addition of antibiotic. The samples were immediately

transferred to RNAprotect Bacteria Reagent (Qiagen) following the supplier's instructions. The MIC was determined using the broth dilution method on 96-well plate in cation-adjusted Mueller-Hinton broth 2 (Sigma-Aldrich).

Microscopy:

S. aureus NCTC8325 cells, grown to 0.3-0.4 OD_{600} in nutrient broth (Oxoid), were treated with MGB-BP-3 (3 µg/ml) for *ca.* 40 mins. DNA binding dye Hoechst 33342 was used as a positive control (at 14 µg/ml) and DMSO was used as a negative control. Brightfield and fluorescent images (UV filter set) were captured using a Nikon TE2000S inverted fluorescence microscopy with IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA).

RNA extraction and library preparation:

Total RNA was extracted using a bacterial RiboPure RNA Purification Kit (AM1925, ThermoFisher Scientific) immediately after samples were collected. In brief, the cells were disrupted mechanically using Zirconia beads, total RNA was then extracted in phenol and purified using glass-fibre filters. Finally, the samples were treated with DNase I according to the manufacturer's instructions. Total RNA was assessed by QuBit® 2.0 Fluorometer (ThermoFisher Scientific) and the associated Qubit RNA assay (Q32852, ThermoFisher Scientific). RNA integrity was confirmed using an Agilent 2100 Bioanalyzer and the associated RNA 6000 pico kit (Agilent Technologies). Samples containing 4.5 µg of total RNA were depleted for ribosomal RNA using a Ribozero rRNA depletion kit (MRZGP126, Cambio), with depletion confirmed by a Bioanalyzer (Agilent). Ion Torrent RNA-Seq library preparation used an Ion total RNA-Seq Kit V2 (4475936, ThermoFisher Scientific) with Ion xpress RNA-

Seq BC 01-16 kit barcodes (4475485, ThermoFisher Scientific) as per supplier's instructions.

Sequencing and data analysis:

The libraries were sequenced using an Ion Torrent PGM (ThermoFisher Scientific) and raw data analysis carried out using the associated Ion Torrent Suite 5.0.2 (ThermoFisher Scientific). Ion PGM template OT2 kits (4480974, ThermoFisher Scientific) were used for emulsion PCR and enrichment steps, with an Ion PGM 200 kit V2 (4482006, ThermoFisher Scientific) for sequencing reactions. Assessment of Ion Sphere Particle quality was undertaken using the Ion Sphere Quality Control Kit (4468656, ThermoFisher Scientific). Triplicate libraries were pooled together to give treated and untreated pools and sequenced initially using Ion 314 chips V2 (4482261, ThermoFisher Scientific). Individual libraries were resequenced using a 318 chip V2 (4484354, ThermoFisher Scientific) to provide technical replicates. All sequence data are deposited on the Sequence Read Archive (SRA) under BioProject: PRJNA603263 (http://www.ncbi.nlm.nih.gov/bioproject/603263). FastQ output files were trimmed (quality score 0.02, discard reads <50 bp in length) and RNA-Seq analysis was carried out using CLC Genomics Workbench version 7.5.1 (Qiagen). The transcriptomics analysis was undertaken using the Empirical Analysis of DGE tool in CLC, with default parameters. The significance of gene expression between the treated and non-treated samples was assessed using Bonferroni adjusted p-value (<0.05) with over two-fold change in expression. Gene comparisons were made using Venn diagrams.

qRT-PCR:

Duplicate samples of total RNA (1 µg) were reverse-transcribed using a qPCRBIO cDNA synthesis kit (PB30.11-02, PCR Biosystems) according to manufacturer's

instructions together with two negative controls (one without reverse transcriptase and another without RNA template). The amount of synthesised cDNA was determined using the QuantiFluor ssDNA system (Promega) with a Qubit® 2.0 Fluorometer (ThermoFisher Scientific) and the samples were stored at -20°C. Primers (Supplementary Table 1) were designed using Primer3 version 0.4.0 software [13, 14] and their concentrations were optimised to decrease primer dimer formation. Amplification reactions were carried out using a 2x qPCRBIO SyGreen Mix Lo-ROX (PB20.11, PCRBiosystems) according to manufacturer's instructions with 1 µl of cDNA as template. The reactions were run on a Corbett Research 6000 instrument. Amplification efficiency and linearity were determined using a dilution series of DNA. Data analysis was performed on Corbett Rotor Gene 6000 software, Microsoft Office Excel and Minitab statistics package.

Phenotypic microarray (PM) analysis:

Biolog Phenotype MicroArray metabolic panels (PM1, PM2A, PM3B) were used to determine the effect of MGB-BP-3 on growth of *S. aureus* NCTC8325 on single carbon or nitrogen sources. Three dilutions of MGB-BP-3 were tested (0.5 x MIC, 0.25 x MIC, 0.125 x MIC). Biolog chemosensitivity panels (PM11C, PM12B) were used in combination with MGB-BP-3 (0.5 x MIC) to evaluate potential synergy between MGB-BP-3 and other antibiotics. Standard Biolog protocols were used with some variances: sodium pyruvate (5mM, Fisher) was used instead of glucose for PM3B, PM11C and PM12B. Cells were first grown to OD_{600} of 0.3-0.5 and then diluted to OD_{600} 0.03 at inoculation. The various dye mixes were tested and dye mix D was selected. All plates were incubated in the OmniLog instrument (Biolog, Inc. USA) at 37°C for 48 hours. Growth data were recorded every 15 minutes and analysed with OmniLog software Parametric v1.3 and Kinetic v1.3 (Biolog, Inc. USA).

Melting curve analysis of dsDNA with MGB-BP-3:

Three DNA-fragments (70-90 bp long) were amplified by PCR using a standard benchtop PCR machine. The PCR reactions contained *S. aureus* NCTC8325 gDNA as a template, primers (Supplementary Table 1) and GoTaq® G2 Flexi DNA polymerase (M7801, Promega). Resulting PCR products were purified on columns (Isolate II PCR Kit (BIO-52059, Bioline)) that are suitable for >50 bp DNA fragments. The purified DNA fragments were then mixed with 9 µg/ml of MGB-BP-3 in water, and the mixture incubated for 15 min in the dark at room temperature before adding 1:1 volume of ready-made PCR mix that contained an intercalating dye (qPCRBIO SyGreen Mix, PB20.11-05, PCR Biosystems), known not to interfere with PCR. Dissociation-characteristics of dsDNA with MGB-BP-3 were measured and the melting curve analysis was performed by raising temperatures sequentially from 50°C to 99°C using a Rotor-Gene 6000 qPCR machine (RCorbett). Drug-free DNA was used as a control. Statistical significance was assessed by student t-test (n=3).

Electrophoretic Mobility-Shift assays (EMSA):

The *pdnaD* and *pmraY* promoter fragments were synthesized by Integrated DNA Technologies (Leuven, Belgium) and sub-cloned into plasmid pSR, as a source of DNA fragments for EMSA and footprinting experiments [15]. EMSA was carried out essentially as detailed by Rossiter *et al*. [16]. Purified AatII-HindIII promoter fragments were end-labelled with [γ-^{32}P]-ATP and approximately 0.2 nM of each fragment was incubated with varying amounts of MGB-BP-3. The final reaction volume (10 µl) contained HEPES glutamate buffer (pH 8.0, 20 mM HEPES, 5 mM $MgCl_2$, 50 mM potassium glutamate, 1 mM DTT, 5% (v/v) glycerol, 0.5 mg/ml BSA) containing 25 µg/ml herring sperm DNA. MGB-BP-3 was incubated with labelled DNA fragments at

room temperature for 15 minutes, after which samples were loaded directly onto a running 6% (w/v) polyacrylamide gel (12 v/cm), containing 2% (v/v) glycerol and 0.25 x TBE (Tris/ borate/ EDTA buffer). Gels were analysed using a Bio-Rad Personal Molecular Imager (PMI) and Quantity One software (Bio-Rad).

DNase I and permanganate footprinting:

DNase I and potassium permanganate footprinting experiments were performed on ^{32}P-end-labelled AatII-BamHI promoter fragments, using protocols described previously [17, 18]. Each reaction mix (20 µl) contained approximately 1.35 nM template DNA in HEPES glutamate buffer, containing 30 µg/ml herring sperm DNA. DNA fragments were incubated with varying concentrations of MGB-BP-3 for 15 minutes at room temperature before footprinting. For potassium permanganate footprint experiments, herring sperm DNA was omitted from the reaction mixture and either *E. coli* RNA polymerase σ^{70} holoenzyme (Epicentre) or *S. aureus* RNA polymerase σ^{A} holoenzyme [19] were used at a final concentration of 50 nM. The products of all footprinting reactions were analysed by denaturing gel electrophoresis and calibrated with Maxam-Gilbert 'G+A' sequencing reactions of the labelled fragment. Gels were quantified using Bio-Rad PMI and Quantity One software.

Evolution of resistance in the laboratory:

A single colony of *S. aureus* (ATCC clone 43300) was transferred to 5 ml L-Broth (LB) and, after overnight incubation at 37^{o} C, 50 µl of the culture was used for serial daily passaging (1/1,000 dilution) in 5 ml LB having either MGB-BP-3 (0.019 µg/ml, 3 replicates), rifampicin (0.4 ng/ml), or no antibiotic (control). After 4 passages (40 generations), concentrations of antibiotics were doubled and passaged daily for 4

further days (40 generations). Aliquots (5 µl) of various 1/10 serial dilutions of the resulting cultures were then spotted in LB-agar plates having either 0.15 µg/ml MGB-BP-3, or 4 ng/ml rifampicin (each corresponding to 2x MIC80), and plates were incubated overnight at 37^{o} C.

Results:

Exposure to MGB-BP-3 elicits significant changes in the *S. aureus* transcriptome

The minimum inhibitory concentration (MIC) of MGB-BP-3 for *S. aureus* NCTC8325 was 0.19 µg/ml (Supplementary Data MIC). Using fluorescence microscopy, bacterial cells exposed to MGB-BP-3 showed clear fluorescence with no intracellular localisation (Figure 1b). Growth of *S. aureus* in the presence of a sub-lethal concentration of MGB-BP-3 (0.5 x MIC) was found to be affected for 100 minutes post-MGB-BP-3 administration (Figure 1c). Therefore, we decided to perform RNA-Seq experiments at an early time-point (ten minutes) after challenge with MGB-BP-3. This transcriptomic analysis identified 698 transcripts showing significant changes (Supplementary Table 2), some of which were confirmed by quantitative RT-PCR (Supplementary Data qRT-PCR). Previous work by Chaudhuri *et al*. had identified 351 essential genes for growth and survival of *S. aureus* in laboratory culture [20]. Out of the total of 404 downregulated genes in cultures treated with MGB-BP-3, 62 had been classified as essential, and belonged to a variety of gene ontology categories (Figure 2).

Further gene ontology analysis of the RNA-Seq data using TIGRFAM protein families [21] identified disruptions in transcription of genes assigned to every Main Role category. Overall, transcription was down-regulated in all categories apart from amino acid biosynthesis, fatty acid and phospholipid metabolism, mobile genetic element functions and transcription (Figures 3 & 4). Transcription of certain non-essential, amino acid biosynthetic genes was stimulated (Supplementary Table 2). Some of these effects were consistent with downregulation of the arginine repressor (SAOUHSC_01617, *argR*) and subsequent consequences on its target genes. Genes involved in biosynthesis of molybdopterin were upregulated, whereas various genes involved in menaquinone and ubiquinone biosynthesis were downregulated significantly (Supplementary Table 2). Some essential genes for pantothenate and coenzyme A biosynthesis and for pyridine nucleotide biosynthesis were downregulated significantly.

Over half of the genes associated with biosynthesis and degradation of the murein sacculus and peptidoglycan were down-regulated significantly in the RNA-Seq experiment. (Supplementary Table 2). Reduced expression of essential cell division genes) was also noted while the septation ring formation regulator (SAOUHSC_01827, *ezrA*) was also repressed. Some genes involved in DNA replication were down-regulated but, by contrast, some genes involved in DNA supercoiling and primosome formation were up-regulated.

Changes such as those described above could reflect either direct interaction of MGB-BP-3 at the level of the individual gene (considered in the section on *dnaD* and *mraY* promoter regions below), or secondary effects via global regulators. This possibility prompted an assessment of the diverse phenotypes of *S. aureus* when challenged with MGB-BP-3.

Conditional essentiality allows correlation between phenotype and transcriptotype.

Omnilog colorimetric redox phenoarrays were used to directly assess the effect of MGB-BP-3 on metabolism. This approach has the advantage of testing conditional essentiality of down-regulated transcripts in the presence of a sole carbon or nitrogen source. Out of the 191 carbon and 95 nitrogen sources tested and in the absence of MGB-BP-3, *S. aureus* NCTC8325 could utilise 63 carbon and 23 sole nitrogen sources (Supplementary Data; Phenoarrays).

When challenged with sub-MIC levels of MGB-BP-3, *S. aureus* showed several regimes concerning the ability to utilise individual substrates for growth, and in a dose-dependent response (0.5 x MIC, 0.25 x MIC, 0.125 x MIC of MGB-BP-3; Supplementary Data Phenoarrays). Growth on thymidine, glutamine, glycine and arginine as a sole carbon source was affected by MGB-BP-3, whereas growth on ornithine as a single carbon source was not. This was consistent with the results of RNA-Seq (see Discussion).

Melting curves of MGB-BP-3 bound to dsDNA reveals upstream binding sites

To further identify putative MGB-BP-3 binding sites, upstream regions of two genes shown to be repressed by RNA-Seq (*dnaD* (*SAOUHSC_01470*) and *mraY* (*SAOUHSC_01146*)) were taken forward for melt analysis after being randomly selected from the ten most down-regulated transcripts. MGB-BP-3 would be expected to bind to such a DNA fragment, stabilise its structure, and increase its melting temperature. The genome locations of both genes showed no other genes located directly upstream from the start of the gene of interest. A GC-rich (GC-content 50%) internal region of *gyrA* (*SAOUHSC_00006*) was used as comparative control. The

melting temperature of each DNA fragment was increased in the presence of MGB-BP-3, indicating binding of the antibiotic to these AT-rich regions (GC-content of 25-26%) (Figure 5). By contrast, the melting temperature of the control fragment (*gyrA* internal region) was unchanged in the presence of MGB-BP-3.

MGB-BP-3 binds to the *dnaD* and *mraY* promoter regions to interfere with transcriptional initiation.

DNA melt curves indicated that MGB-BP-3 binds to DNA with some specificity. To examine this further, EMSA assays investigated the binding of MGB-BP-3 to end-labelled DNA fragments, which carried the *dnaD* and *rmaY* promoter regions (*pdnaD* and *pmraY*) (Figure 6). These were chosen as representative of genes that were shown to be down-regulated on challenge with MGB-BP-3 by RNA-Seq and qPCR.

MGB-BP-3 is a small molecule (MW 746 Da). A ladder of fragments with altered mobility was observed for each, indicating that each promoter fragment carried multiple binding sites for MGB-BP-3 (Figure 7). Thus, to pinpoint these MGB-BP-3 binding sites at high resolution, DNase I footprinting was used (Figure 8). For each promoter fragment, discrete high affinity binding sites were observed at low concentrations of MGB-BP-3, whilst additional protections from DNase I were observed as the concentration of antibiotic was increased. In particular, high affinity binding sites for MGB-BP-3 correlated with regions of A/T richness, close to the experimentally determined transcription start sites and overlapping the predicted -10 elements of each promoter (Figures 6 and 8) [27-29]. As positioning of such high affinity binding sites for MGB-BP-3 could interfere with transcriptional initiation, potassium permanganate footprinting was used to examine promoter isomerisation by RNA polymerase at each promoter. Single-stranded DNA, generated by DNA melting

during transcriptional initiation, is sensitive to modification by permanganate, which can be detected by gel electrophoresis [18, 30]. In the absence of MGB-BP-3, both *E. coli* and *S. aureus* RNA polymerase holoenzyme could recognise *pdnaD* and *pmraY*, unwinding the DNA surrounding the transcription start site and each -10 promoter element. However, in the presence of MGB-BP-3, unwinding around the promoter was completely inhibited (Figures 9 and 10), confirming that MGB-BP-3 prevents transcription initiation at *pdnaD* and *pmraY* by occluding the promoter region and preventing RNA polymerase isomerisation.

Can *S. aureus* evolve to be resistant to MGB-BP-3?

As MGB-BP-3 binds to multiple sites in the bacterial genome, with consequential reduction in transcription of essential genes, a great number of mutations would have to occur within a short time period to result in resistance to this antibiotic. This contrasts with other antibiotics such as rifampicin, which bind to a single target, such that a single genetic mutation is sufficient to confer resistance. Indeed, after serial passaging of three independent populations of *S. aureus* at sub-MIC80 concentrations of MGB-BP-3 (up to 0.5x MIC80) for 80 generations, no resistant clone was isolated. By contrast, serial passaging for the same period at sub-MIC80 concentrations of rifampicin (up to 0.4x MIC80), resistant colonies were identified even at the lowest spotted concentration (corresponding to OD_{600} 10^{-8}, Supplementary Data; Resistance). In a second experiment, a population of *S. aureus* was passaged at 0.66x MIC80 of MGB-BP-3 for 280 generations, but no resistance was observed (data not shown).

Discussion:

DNA minor groove-binding drugs have shown promise in the treatment of a variety of diseases [31]. Amongst these, novel antibiotic MGB-BP-3 is in development for treatment of *Clostridioides difficile* infections and has completed Phase II clinical trials successfully. Here, *S. aureus* was used as a model organism to study MGB-BP-3 mode of action, since much of the preliminary work for the MGB-BP-3 development program had already been done with this organism. Moreover, this choice simplified the growth conditions for the Biolog phenotypic array work.

Transcriptomics identified 62 essential genes that were repressed in the presence of this DNA-binding antibiotic (Figure 2). Transcriptional changes in seven of these genes (*argH, cdd, citC, gapA, hup, mvaK2* and *pyrF*) was confirmed independently by qRT-PCR. Several genes with regulatory functions that could account for some of the changes seen in the RNA-Seq data had expression altered significantly on exposure to MGB-BP-3. (Supplementary Table 2). For instance, anti-anti-sigma factor *rsbV* (SAOUHSC_02300) was repressed whereas RNA polymerase sigma factor RpoD coded by *sigA* (SAOUHSC_01662) was stimulated. Moreover, two-component systems such as *walKR* (SAOUHSC_00021 and SAOUHSC_00020) and *phoPR* (SAOUHSC_01800 and SAOUHSC_01799), together with glutamine synthetase repressor *glnR* (SAOUHSC_01285), DNA-binding response regulator *srrA* (SAOUHSC_01586), transcriptional regulator *nrdR* (SAOUHSC_01793) and transcription of LexA repressor (SAOUHSC_01333, *lexA*) were down-regulated significantly.

Microtiter plate-based phenoarrays offer an alternative strategy to investigate challenge of bacterial cells by MGB-BP-3, illustrating the conditional essentiality

effects of the antibiotic and providing data (Supplementary Data Phenoarrays) to compare with the transcriptomic response. Thus, none of the genes for ornithine metabolism (SAOUHSC_00076, SAOUHSC_00148, SAOUHSC_00150, SAOUHSC_00894, SAOUHSC_01128, SAOUHSC_02967, SAOUHSC_02968) had significantly altered expression profiles on challenge by MGB-BP-3, consistent with the data from the phenotypic arrays. Although arginine is a precursor of ornithine [25], its metabolism was affected negatively on challenge by MGB-BP-3 in a dose dependent manner. Transcriptomics revealed that expression of *ahrC* (SAOUHSC_01617) was significantly downregulated (by 6-fold) on exposure to MGB-BP-3. AhrC acts as a repressor of arginine biosynthesis and an activator of the arginine catabolic pathway in *B. subtilis* [26]. Thus down-regulation of arginine catabolism directly could explain the differences seen in response to MGB-BP-3 for growth on either arginine or ornithine as a single carbon source. Further correlations between the transcriptomic data and phenotypic arrays reinforced the multiplicity of effects on the bacterial cells on challenge with MGB-BP-3. Taken together, our data supports the hypothesis that MGB-BP-3 binds to and inhibits multiple essential promoters on the *S. aureus* chromosome.

Reflecting the current annotation status of *S. aureus,* genes encoding hypothetical proteins and proteins with unknown function were the largest category in the transcriptomic data that showed a general trend of transcriptional repression. By contrast, hypothetical protein SAOUHSC_00420 was the most upregulated gene (203-fold) in the RNA-Seq dataset (Figure 4). It is predicted to encode a sodium-dependent transporter that has been implicated in extracellular DNA release during biofilm formation in *S. aureus* [24]. The second most enhanced gene *SAOUHSC_00880* (53-fold change) was a hypothetical protein, also assigned to the category of transport and

binding proteins. A further 7 out of 16 of the most upregulated genes belonged to this category, which may reflect a generalised antibiotic stress response.

Direct inhibition of transcription at individual gene loci by was investigated further by focussing on two essential genes as exemplars, *dnaD* (SAOUHSC_01470) and *mraY* (SAOUHSC_01146). DNA melt curves demonstrated binding of MGB-BP-3 to upstream regions of these genes (Figure 5), which was confirmed by EMSA (Figure 7). Much higher resolution of MGB-BP-3 binding upstream of these genes was determined with DNAse I footprinting assays (Figures 6 and 8) demonstrating that high affinity binding sites for MGB-BP-3 overlap both the *dnaD* and *mraY* promoters. The presence of multiple MGB-BP-3 binding sites upstream of both *dnaD* and *mraY* may explain the strength of the inhibitory effect at these particular promoters (Figures 6 and 8). Permanganate footprinting of these promoters in the presence of purified RNA polymerase (RNAP) from both *E. coli* and *S. aureus* demonstrated that MGB-BP-3 binding to the -10 element inhibited the isomerisation of the promoter by RNAP holoenzyme (core polymerase plus SigA, Figures 6, 9 and 10). Specificity of transcription from promoters such as *dnaD* and *mraY* is conferred by the SigA subunit of RNAP. Thus, it has been demonstrated that MGB-BP-3 binds to certain SigA-dependent promoter regions, preventing transcription of these genes. This is almost certainly not the sole mechanism of action of MGB-BP-3 since transcription is not the only essential protein-DNA interaction, but is sufficient across the various target genes to explain the catastrophic death on the bacterial cells on challenge with MGB-BP-3.

Microbial drug resistance has become a global health problem and the need to bring new antibiotics to the market is increasing [32]. Resistance against new drugs can develop within the first couple of years of the drug entering the market [33]. Our data

suggests that MGB-BP-3 binds to multiple sites on *S. aureus* chromosome, making it less likely for resistance to evolve by drug target mutation. To test this hypothesis, laboratory-directed evolution experiments using sub-inhibitory concentrations of MGB-BP-3 showed no resistance in *S. aureus*. In contrast, resistance to the single target RNA-polymerase inhibitor rifampicin arose rapidly (Supplementary Data; Resistance).

In summary, a novel mode of action for MGB-BP-3 against *S. aureus* has been demonstrated by thorough investigation of two exemplar drug-binding sites on the *S. aureus* chromosome. RNA-Seq analysis revealed a total of 698 transcripts with drug altered expression profiles. It is, therefore, highly likely that there are further multiple MGB-BP-3 binding sites on the *S. aureus* chromosome. Further analysis of DNA binding site sequences combined with rational design of other antibiotic MGBs could provide a powerful methodology for new drug development.

References:

1. Barrett, M. P., Gemmell, C. G. & Suckling, C. J. Minor groove binders as anti-infective agents. *Pharmacol. Ther.* **139**, 12-23 (2013).

2. Ross, W., Ernst, A. & Gourse, R. L. Fine structure of E. coli RNA polymerase-promoter interactions: alpha subunit binding to the UP element minor groove. *Genes Dev.* **15**, 491-506 (2001).

3. Cai, X., Gray, P. J., Jr. & Von Hoff, D. D. DNA minor groove binders: back in the groove. *Cancer Treat. Rev.* **35**, 437-450 (2009).

4. Puschendorf, B. *et al.* Studies on the effect of distamycin A on the DNA dependent RNA polymerase system. *Biochem. Biophys. Res. Commun.* **43**, 617-624 (1971).

5. Taylor, A., Webster, K. A., Gustafson, T. A. & Kedes, L. The anti-cancer agent distamycin A displaces essential transcription factors and selectively inhibits myogenic differentiation. *Mol. Cell. Biochem.* **169**, 61-72 (1997).

6. Anthony, N. G. *et al.* Antimicrobial lexitropsins containing amide, amidine, and alkene linking groups. *J. Med. Chem.* **50**, 6116-6125 (2007).

7. Hlaka, L. *et al.* Evaluation of minor groove binders (MGBs) as novel anti-mycobacterial agents and the effect of using non-ionic surfactant vesicles as a delivery system to improve their efficacy. *J. Antimicrob. Chemother.* **72**, 3334-3341 (2017).

8. Scott, F. J. *et al.* Selective anti-malarial minor groove binders. *Bioorg. Med. Chem. Lett.* **26**, 3326-3329 (2016).

9. Scott, F. J. *et al.* An evaluation of Minor Groove Binders as anti-Trypanosoma brucei brucei therapeutics. *Eur. J. Med. Chem.* **116**, 116-125 (2016).

10. Scott, F. J. *et al.* An evaluation of Minor Groove Binders as anti-fungal and anti-mycobacterial therapeutics. *Eur. J. Med. Chem.* **136**, 561-572 (2017).

11. Scott, F. J. *et al.* An evaluation of Minor Groove Binders as anti-lung cancer therapeutics. *Bioorg. Med. Chem. Lett.* **26**, 3478-3486 (2016).

12. Anthony, N. G. *et al.* Short lexitropsin that recognizes the DNA minor groove at 5'-ACTAGT-3': understanding the role of isopropyl-thiazole. *J. Am. Chem. Soc.* **126**, 11338-11349 (2004).

13. Koressaar, T. & Remm, M. Enhancements and modifications of primer design program Primer3. *Bioinformatics* **23**, 1289-1291 (2007).

14. Untergasser, A. *et al.* Primer3--new capabilities and interfaces. *Nucleic Acids Res.* **40**, e115 (2012).

15. Kolb, A., Kotlarz, D., Kusano, S. & Ishihama, A. Selectivity of the Escherichia coli RNA polymerase E sigma 38 for overlapping promoters and ability to support CRP activation. *Nucleic Acids Res.* **23**, 819-826 (1995).

16. Rossiter, A. E. *et al.* Expression of different bacterial cytotoxins is controlled by two global transcription factors, CRP and Fis, that co-operate in a shared-recruitment mechanism. *Biochem. J.* **466**, 323-335 (2015).

17. Browning, D. F. *et al.* The Escherichia coli K-12 NarL and NarP proteins insulate the nrf promoter from the effects of integration host factor. *J. Bacteriol.* **188**, 7449-7456 (2006).

18. Squire, D. J. *et al.* Competition between NarL-dependent activation and Fis-dependent repression controls expression from the Escherichia coli yeaR and ogt promoters. *Biochem. J.* **420**, 249-257 (2009).

19. Reynolds, J. & Wigneshweraraj, S. Molecular insights into the control of transcription initiation at the Staphylococcus aureus agr operon. *J. Mol. Biol.* **412**, 862-881 (2011).

20. Chaudhuri, R. R. *et al.* Comprehensive identification of essential Staphylococcus aureus genes using Transposon-Mediated Differential Hybridisation (TMDH). *BMC Genomics* **10**, 291 (2009).

21. Fuchs, S. *et al.* AureoWiki The repository of the Staphylococcus aureus research and annotation community. *Int. J. Med. Microbiol.* **308**, 558-568 (2018).

22. Smits, W. K., Merrikh, H., Bonilla, C. Y. & Grossman, A. D. Primosomal proteins DnaD and DnaB are recruited to chromosomal regions bound by DnaA in Bacillus subtilis. *J. Bacteriol.* **193**, 640-648 (2011).

23. Huang, Y. H., Lien, Y., Huang, C. C. & Huang, C. Y. Characterization of Staphylococcus aureus Primosomal DnaD Protein: Highly Conserved C-Terminal Region Is Crucial for ssDNA and PriA Helicase Binding but Not for DnaA Protein-Binding and Self-Tetramerization. *PLoS One* **11**, e0157593 (2016).

24. DeFrancesco, A. S. *et al.* Genome-wide screen for genes involved in eDNA release during biofilm formation by Staphylococcus aureus. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E5969-E5978 (2017).

25. Nuxoll, A. S. *et al.* CcpA regulates arginine biosynthesis in Staphylococcus aureus through repression of proline catabolism. *PLoS Pathog.* **8**, e1003033 (2012).

26. Miller, C. M., Baumberg, S. & Stockley, P. G. Operator interactions by the Bacillus subtilis arginine repressor/activator, AhrC: novel positioning and DNA-mediated assembly of a transcriptional activator at catabolic sites. *Mol. Microbiol.* **26**, 37-48 (1997).

27. Prados, J., Linder, P. & Redder, P. TSS-EMOTE, a refined protocol for a more complete and less biased global mapping of transcription start sites in bacterial pathogens. *BMC Genomics* **17**, 849 (2016).

28. Choe, D. *et al.* Genome-scale analysis of Methicillin-resistant Staphylococcus aureus USA300 reveals a tradeoff between pathogenesis and drug resistance. *Sci. Rep.* **8**, 2215 (2018).

29. Browning, D. F. & Busby, S. J. Local and global regulation of transcription initiation in bacteria. *Nat. Rev. Microbiol.* **14**, 638-650 (2016).

30. Sasse-Dwight, S. & Gralla, J. D. Probing co-operative DNA-binding in vivo. The lac O1:O3 interaction. *J. Mol. Biol.* **202**, 107-119 (1988).

31. Rahman, A., O'Sullivan, P. & Rozas, I. Recent developments in compounds acting in the DNA minor groove. *MedChemComm* **10**, 26-40 (2019).

32. Theuretzbacher, U. *et al.* Analysis of the clinical antibacterial and antituberculosis pipeline. *Lancet Infect. Dis.* **19**, e40-e50 (2019).

33. Coates, A. R., Halls, G. & Hu, Y. Novel classes of antibiotics or more of the same? *Br. J. Pharmacol.* **163**, 184-194 (2011).

Acknowledgements

LK was supported by BBSRC grant BB/K019600/1 to NPT, ISH and CJS. Work in NPT's laboratory is also supported by BBSRC grants BB/V509243/1 and BB/S507106/1. KL was supported by CSO grant TCS/16/24 to ISH, CJS and NPT. DFB was supported by BBSRC grant BB/R017689/1. We thank Ramesh Wigneshweraraj and Lynn Burchell for kindly providing purified *S. aureus* core RNA polymerase and σ^A protein.

Author Contributions

NPT conceived all experiments, LK conceived and performed all microscopy, RNA-sequencing, qRT-PCR, melting analysis and phenotypic microarray experiments, DFB conceived and performed all the EMSA, DNase I and permanganate footprinting experiments, KL performed evolution of resistance experiment and TS performed independent growth studies using single carbon source, LK and NPT

analysed data and wrote the draft manuscript. DFB, KL, ISH and CJS analysed data and contributed to the manuscript. NPT, ISH and CJS acquired funding.

Competing Interests statement

The authors declare no competing interests.

Figures

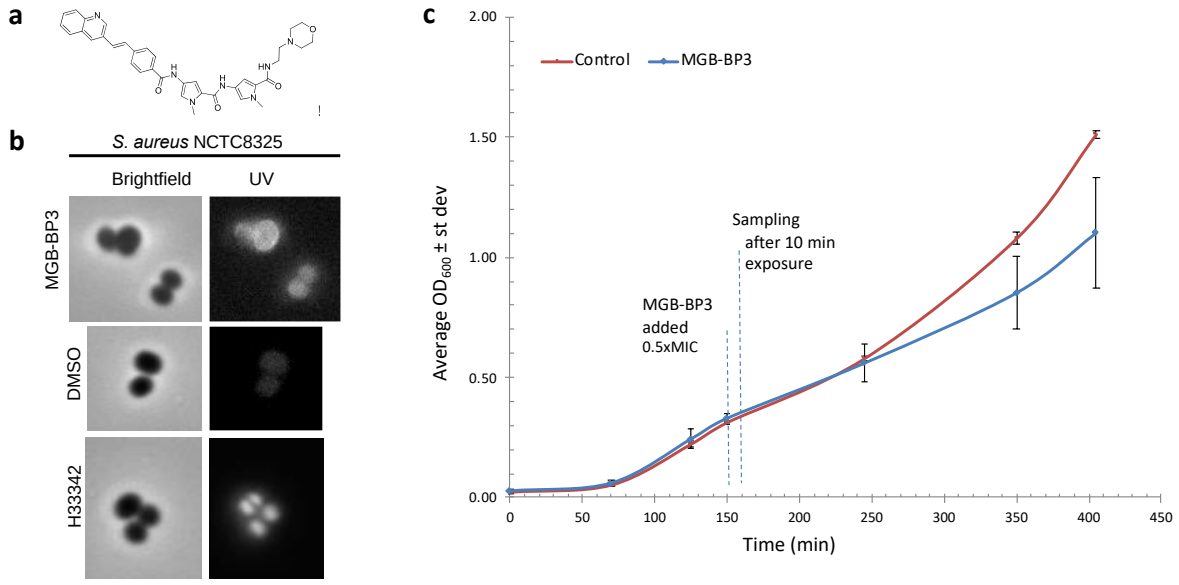


Figure 1: Sub-lethal exposure of *S. aureus* to MGB-BP-3. **a**. Structure of MGB-BP-3. **b**. Microscopy images showing binding of MGB-BP-3 to *S. aureus* cells under UV filter set. DNA binding dye, H33342, used as a positive control and DMSO as a negative control. **c**. *S. aureus* NCTC8325 growth, as measured by OD_{600} MGB-BP-3 (0.5 x MIC) treated and untreated (control) cultures. Cells were harvested for RNA-Seq experiment 10 min after exposure. Error bars, s.d., $n = 3$

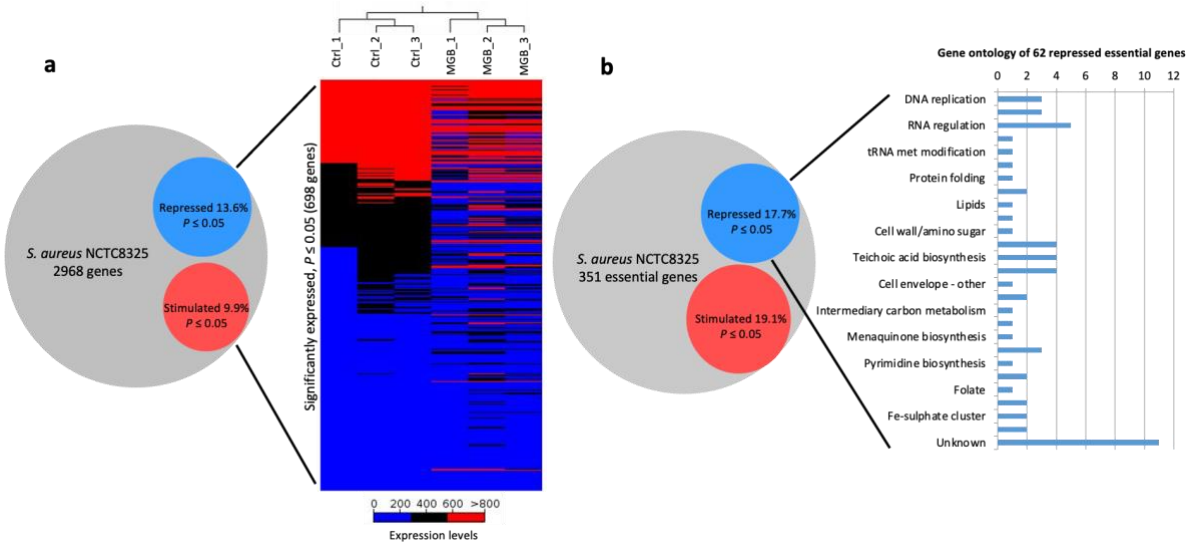
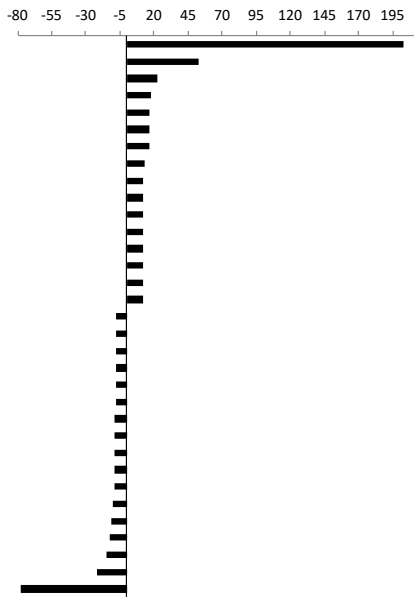


Figure 2: MGB-BP-3 induced transcriptional changes to *S. aureus* NCTC8325 transcriptome on challenge with 0.095 μg/ml MGB-BP-3 (0.5 X MIC) **a**. Venn diagram of RNA-Seq Bonferroni corrected p-values (< 0.05) and a heat map showing hierarchical clustering of the six RNA-Seq samples (3 x ctrl and 3 x drug treated) with a total of 698 significantly expressed genes. **b**. Gene ontology of 62 essential *S. aureus* NCTC8325 genes that were identified in RNA-Seq experiment to be downregulated on challenge with MGB-BP-3.

Main role	Total no. of genes in each category	Percentage of genes differentially regulated	
		Stimulated	Repressed
Amino acid biosynthesis	74	12.2	6.8
Biosynthesis of cofactors, prosthetic groups, and carriers	120	12.5	15.0
Cell envelope	72	16.7	23.6
Cellular processes	142	7.0	19.0
Central intermediary metabolism	37	10.8	21.6
DNA metabolism	125	12.0	14.4
Energy metabolism	146	7.5	10.3
Fatty acid and phospholipid metabolism	27	37.0	7.4
Mobile and extrachromosomal element functions	45	11.1	6.7
Protein fate	117	8.5	13.7
Protein synthesis*	104	8.7	12.5
Purines, pyrimidines, nucleosides, and nucleotides	60	10.0	10.0
Regulatory functions	90	7.8	27.8
Signal transduction	39	2.6	35.9
Transcription	32	9.4	6.3
Transport and binding proteins	234	12.0	16.7
Hypothetical protein/unknown function	1484	8.0	13.1
*Excluding ribosomal proteins			

Figure 3: Gene ontology analysis of the *S. aureus* transcriptome after challenge by MGB-BP-3. The percentage of significantly expressed genes belonging to different TIGRFAM protein families is illustrated. The data excludes ribosomal proteins.



Gene ID	Gene symbol	Product	TIGRFAM Main Role	Fold change
SAOUHSC_00420	-	hypothetical protein	-	203
SAOUHSC_00880	-	hypothetical protein	Transport and binding proteins	53
SAOUHSC_02184	-	phi PVL orf 14-like protein	-	22
SAOUHSC_01843	isdH	hypothetical protein	-	17
SAOUHSC_00168	-	hypothetical protein	Transport and binding proteins	17
SAOUHSC_00302	-	hypothetical protein	-	17
SAOUHSC_00315	mepA	hypothetical protein	Transport and binding proteins	17
SAOUHSC_00899	argG	argininosuccinate synthase	Amino acid biosynthesis	13
SAOUHSC_03054	rnpA	ribonuclease P	Transcription	13
SAOUHSC_01346	opuD1	glycine betaine transporter	Transport and binding proteins	12
SAOUHSC_02187	-	HK97 family phage protein	Mobile and extrachromosomal element functions	12
SAOUHSC_00169	-	peptide ABC transporter permease	Transport and binding proteins	12
SAOUHSC_02322	-	hypothetical protein	Unknown function	12
SAOUHSC_02108	ftnA	ferritin	Transport and binding proteins	12
SAOUHSC_00928	opp-4A	oligopeptide ABC transporter substrate-binding protein	Transport and binding proteins	12
SAOUHSC_00691	uppP	undecaprenyl pyrophosphate phosphatase	Cell envelope	12
SAOUHSC_02473	-	hypothetical protein	-	-8
SAOUHSC_01356	glcT	transcription antiterminator	Biosynthesis of cofactors, prosthetic groups, and carriers	-8
SAOUHSC_01706	-	hypothetical protein	-	-8
SAOUHSC_00740	-	hypothetical protein	Transport and binding proteins	-8
SAOUHSC_01142	mraZ	cell division protein MraZ	Regulatory functions; Cellular processes	-8
SAOUHSC_02100	vraT	hypothetical protein	-	-8
SAOUHSC_01144	ftsL	cell division protein	Cellular processes	-9
SAOUHSC_A01723	-	hypothetical protein	-	-9
SAOUHSC_01039	-	hypothetical protein	Cellular processes; DNA metabolism	-9
SAOUHSC_01143	mraW	S-adenosyl-methyltransferase MraW	Protein synthesis	-9
SAOUHSC_02309	-	hypothetical protein	-	-9
SAOUHSC_02846	-	hypothetical protein	Unknown function	-10
SAOUHSC_00824	-	hypothetical protein	-	-11
SAOUHSC_00979	-	hypothetical protein	Protein synthesis	-12
SAOUHSC_02727	-	hypothetical protein	-	-15
SAOUHSC_02931	-	hypothetical protein	-	-22
SAOUHSC_00593	-	hypothetical protein	-	-78

Figure 4: List of genes with the largest fold-changes of the *S. aureus* transcriptome on challenge by MGB-BP-3. The top 10 of genes with the smallest or largest fold-changes are included.

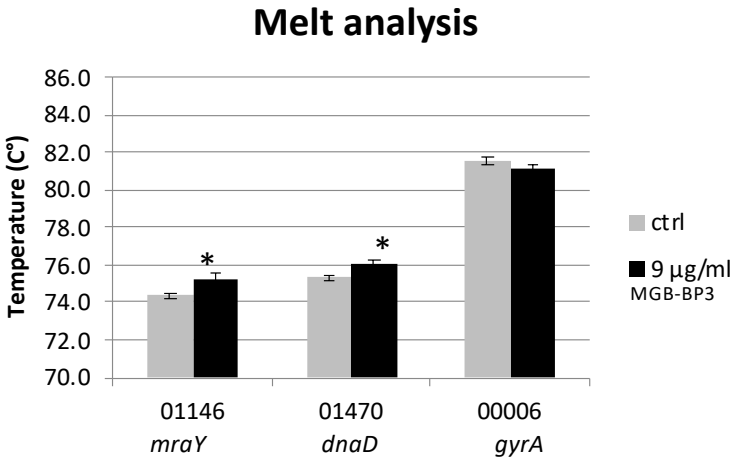


Figure 5: Melt analysis of DNA-MGB-BP-3 (9 µg/ml) complexed with promoter segments of *mraY* and *dnaD*. An AT-rich internal region of *gyrA* was used as DNA segment that should have no binding site for MGB-BP-3. Control samples (grey) did not contain MGB-BP-3. Statistical significance ($p \leq 0.05$) is indicated with an asterisk. Error bars, s.d., $n = 3$

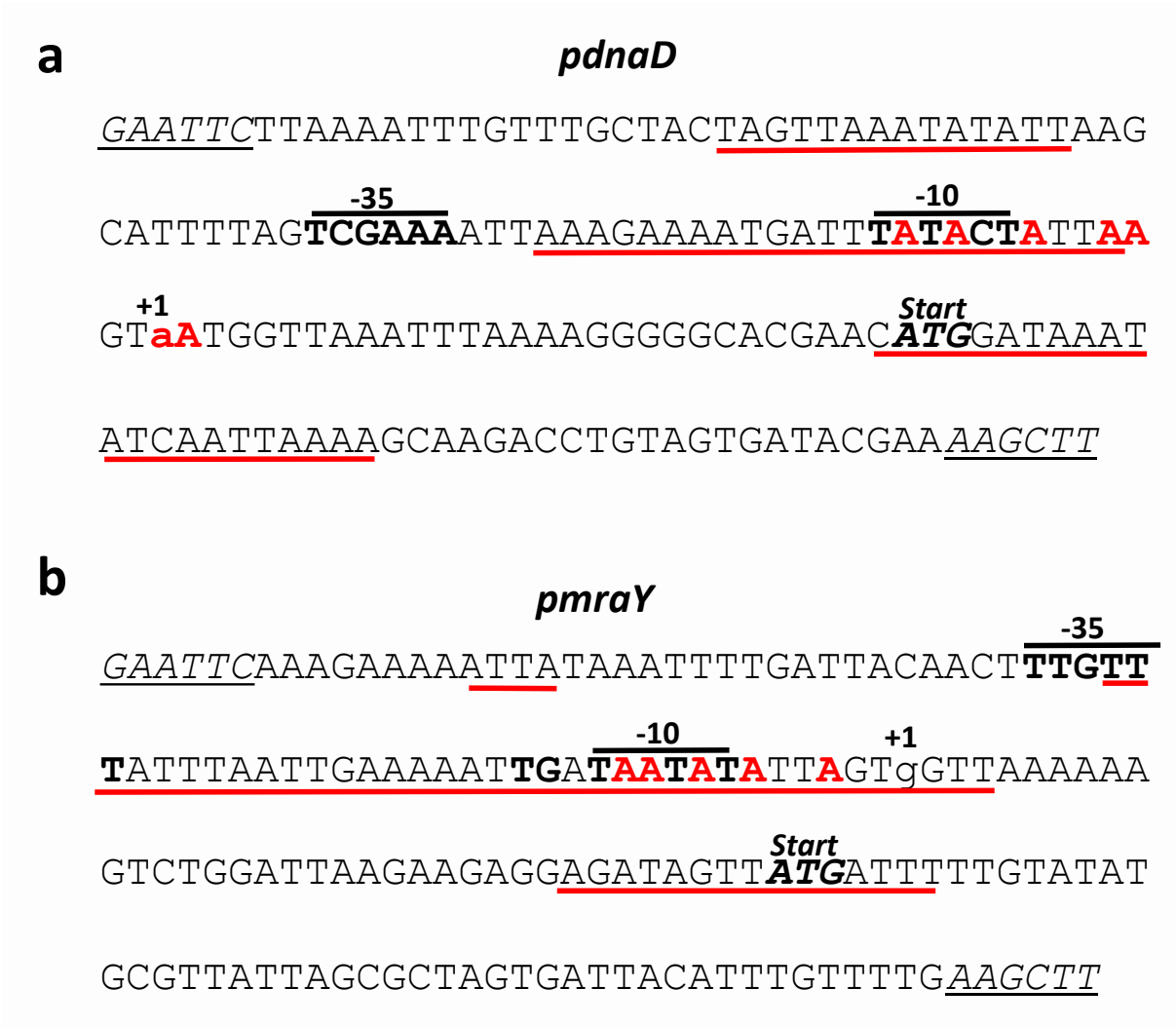


Figure 6: Organisation of the *pdnaD* (a) and *prmaY* (b) promoter regions of *S. aureus*. (+1 and lower case) denotes the experimentally determined transcription start site [27, 28] and the predicted -10 and -35 regions are also shown in bold [29]. The cleavage sites produced by potassium permanganate footprinting are shown in red and bold, whilst the extent of the high affinity MGB-BP-3 binding sites, determined by DNase I footprinting, are underlined in red. The translation start of each gene (ATG) is italicised and bold. The EcoRI and HindIII sites introduced onto each fragment to facilitate cloning into pSR are shown italicised and underlined.

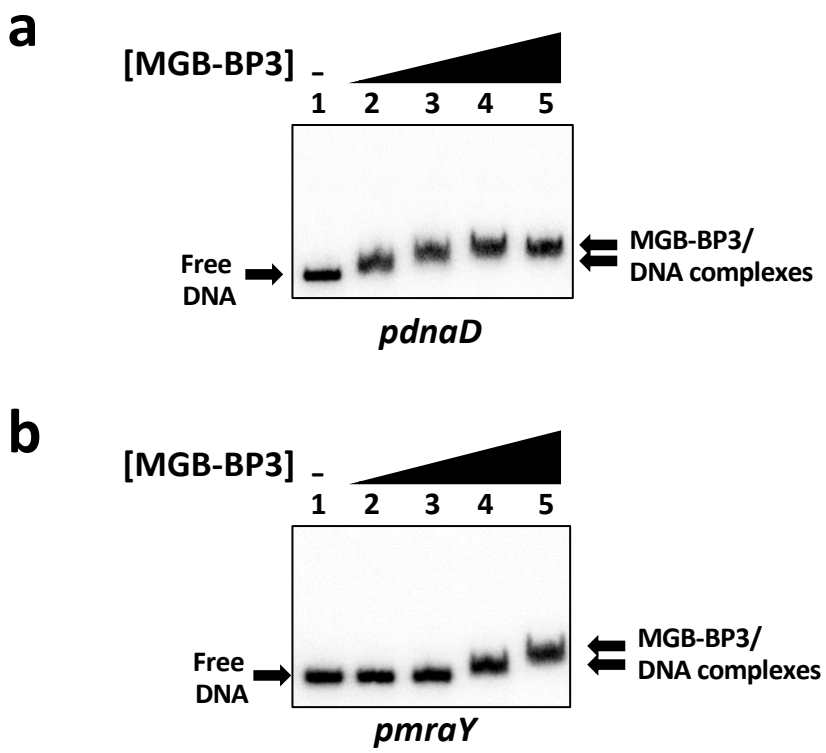


Figure 7: MGB-BP-3 binds to the *pdnaD* and *prmaY S. aureus* promoters. EMSA of the (a) *pdnaD* and (b) *pmraY* promoter regions with MGB-BP-3. End-labelled AatII-HindIII fragments were incubated with increasing concentrations of MGB-BP-3 as follows: lane 1, no MGB-BP-3; lane 2, 1.25 µg/ml; lane 3, 2.5 µg/ml; lane 4, 5 µg/ml; lane 5, 10 µg/ml.

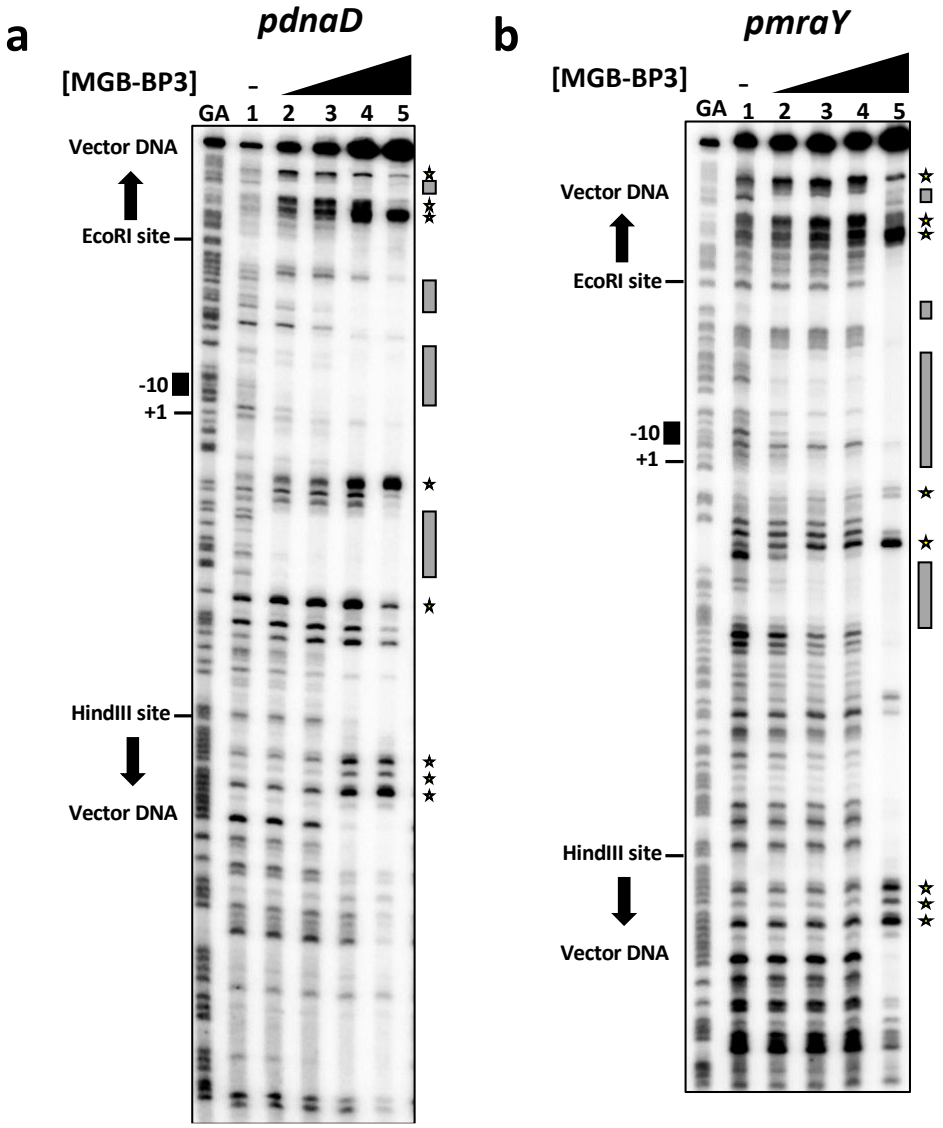


Figure 8: MGB-BP-3 binds to discrete sites within the *pdnaD* and *prmaY S. aureus* promoters. DNase I footprinting analysis of the (a) *pdnaD* and (b) *pmraY* promoter regions when bound by MGB-BP-3. End-labelled AatII-BamHI fragments were incubated with increasing concentrations of MGB-BP-3 and subjected to DNase I footprinting. The concentrations of MGB-BP-3 used were as follows: lane 1, no MGB-BP-3; lane 2, 1.25 µg/ml; lane 3, 2.5 µg/ml; lane 4, 5 µg/ml; lane 5, 10 µg/ml. Gels were calibrated using Maxam-Gilbert 'G+A' sequencing reactions (lane GA) and the location of the -10 element and transcription start site (+1) for each promoter is indicated. Regions of high affinity MGB-BP-3 protection are indicated by grey boxes and hypersensitive sites produced by MGB-BP-3 binding are labelled with stars. The locations of the EcoRI and HindIII sites and the pSR vector sequences on each AatII-BamHI fragment are also marked.

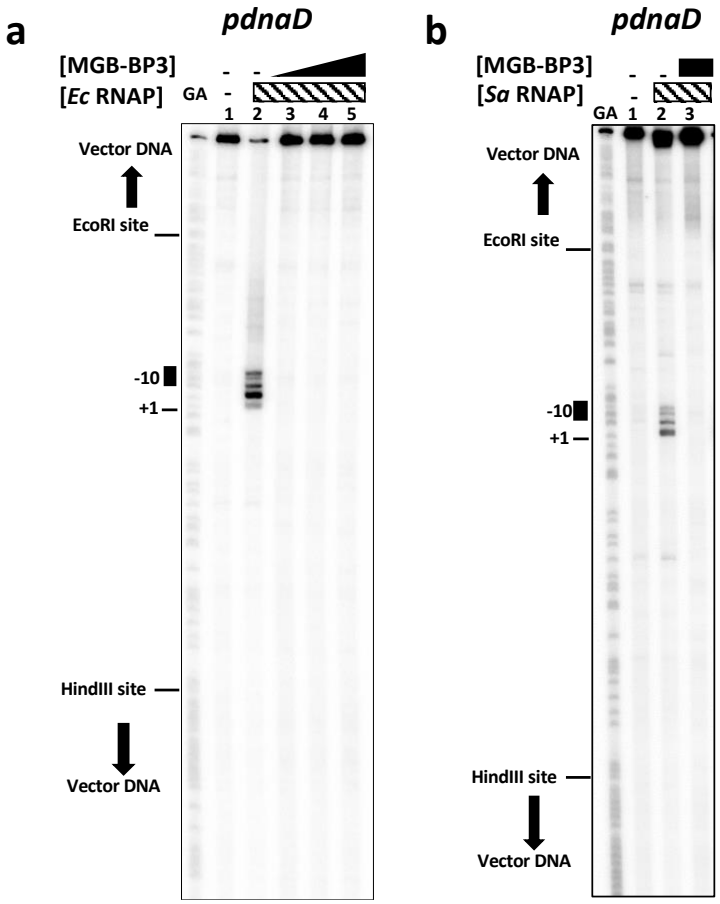


Figure 9: MGB-BP-3 prevents promoter unwinding by both *E. coli* and *S. aureus* RNA polymerase holoenzyme at the *pdnaD S. aureus* promoter. Potassium permanganate footprinting analysis of the *pdnaD* promoter region when bound by MGB-BP-3. (a) End-labelled AatII-BamHI *pdnaD* promoter fragment was pre-incubated with MGB-BP-3, challenged with 50 nM *E. coli* RNA polymerase holoenzyme and subjected to potassium permanganate footprinting. The concentrations of MGB-BP-3 were as follows: lanes 1 and 2, no MGB-BP-3; lane 3, 1.25 µg/ml; lane 4, 2.5 µg/ml; lane 5, 5 µg/ml. (b) End-labelled AatII-BamHI *pdnaD* promoter fragment was pre-incubated with MGB-BP-3, challenged with 50 nM *S. aureus* RNA polymerase holoenzyme, saturated with σ^A, and subjected to potassium permanganate footprinting. The concentrations of MGB-BP-3 were as follows: lanes 1 and 2, no MGB-BP-3; lane 3, 2.5 µg/ml. Gels were calibrated using Maxam-Gilbert 'G+A' sequencing reactions (lane GA) and the location of the predicted *pdnaD* -10 element and experimentally determined transcription start site (+1) is indicated [28]. The positions of the EcoRI and HindIII sites and the pSR vector sequences on each AatII-BamHI fragment are also marked.

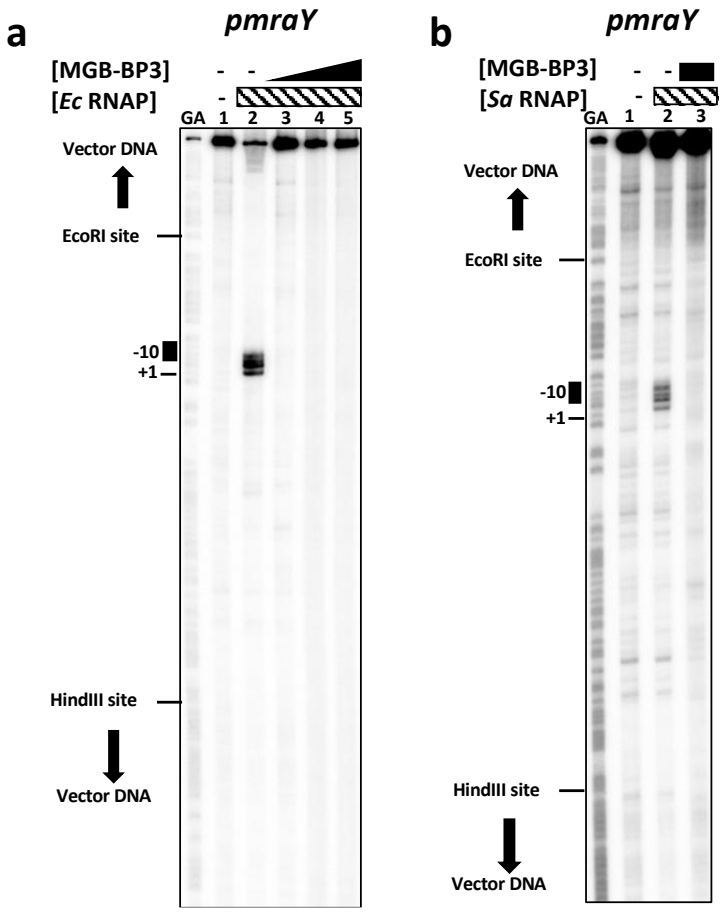
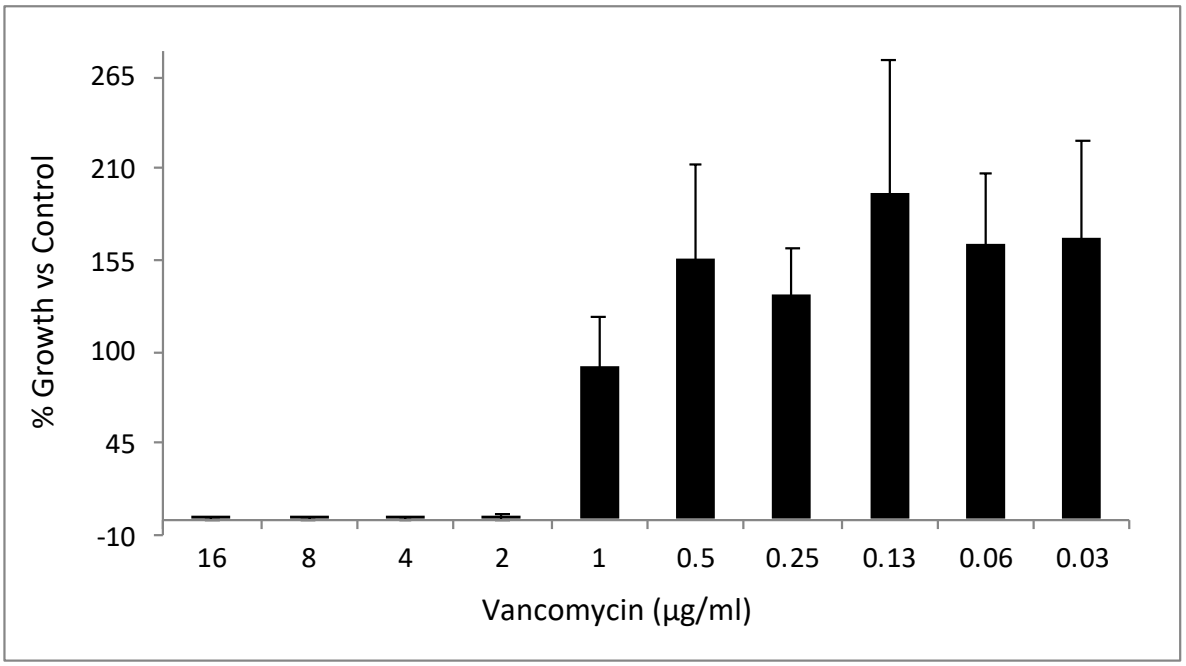
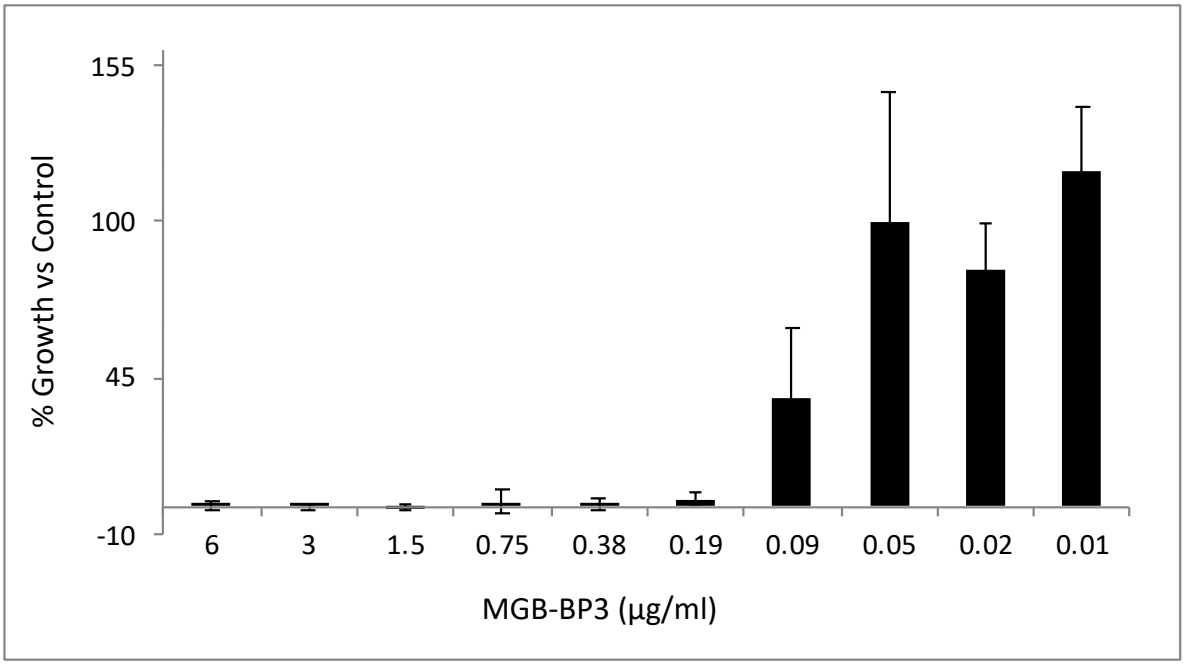


Figure 10: MGB-BP-3 prevents promoter unwinding by both *E. coli* and *S. aureus* RNA polymerase holoenzyme at the *pmraY S. aureus* promoter region. Potassium permanganate footprinting analysis of the *pmraY* promoter region when bound by MGB-BP-3. (a) End-labelled AatII-BamHI *pmraY* promoter fragment was pre-incubated with MGB-BP-3, challenged with 50 nM *E. coli* RNA polymerase holoenzyme and subjected to potassium permanganate footprinting. The concentrations of MGB-BP-3 were as follows: lanes 1 and 2, no MGB-BP-3; lane 3, 1.25 µg/ml; lane 4, 2.5 µg/ml; lane 5, 5 µg/ml. (b) End-labelled AatII-BamHI *pmraY* promoter fragment was pre-incubated with MGB-BP-3, challenged with 50 nM *S. aureus* RNA polymerase holoenzyme, saturated with σ^A, and subjected to permanganate footprinting. The concentrations of MGB-BP-3 were as follows: lanes 1 and 2, no MGB-BP-3; lane 3, 2.5 µg/ml. Gels were calibrated using Maxam-Gilbert 'G+A' sequencing reactions (lane GA) and the predicted location of the *pmraY* -10 element and the experimentally determined transcription start site (+1) is indicated [27]. The positions of the EcoRI and HindIII sites and the pSR vector sequences on each AatII-BamHI fragment are also marked.

Supplementary information

Supplementary Data –MIC. Comparison of the MIC of vancomycin and MGB-BP-3 against *S. aureus*





Supplementary Data – qRT-PCR. Confirmation of selected transcriptional effects of MGB-BP-3 by qRT-PCR.

			RNA-seq.				qRT-PCR			
			EDGE CLC				qRT-PCR (normalized to the amount of total RNA)			
qRT-PCR ID:	Enzyme:	Gene ID:	Expression ctrl	Expression MGB	Bonferroni corrected p-value	fold change	copies (ctrl)	copies (MGB)	p-value (two sample t-test)	fold change
1	Glyceraldehyde 3-phosphate dehydrogenase, type I	SAOUHSC_00795	9,838	24,457	5.75E-07	2.5	1,527,412	2,153,587	0.020	0.5
2	Arginosuccinate lyase	SAOUHSC_00898	29	141	1.59E-16	4.9	9,513	21,782	0.006	1.2
3	Isocitrate dehydrogenase	SAOUHSC_01801	4,394	957	5.24E-05	-4.6	1,062,335	156,196	0.000	-2.8
4	phosphomevalonate kinase	SAOUHSC_00579	166	164	1	-1.0	14,941	9,666	0.015	-0.6
5	Orotidine 5'-phosphate decarboxylase	SAOUHSC_01171	66	215	0.010	3.2	31,934	59,468	0.186	0.9
6	Cytidine deaminase	SAOUHSC_01670	222	34	2.67E-11	-6.5	176,829	21,728	0.000	-3.0
7	DNA-binding protein HU	SAOUHSC_01490	20,341	25,397	1	1.2	6,446,907	6,801,355	0.692	0.1
							*1 µg of total RNA used in each cDNA synthesis			

Supplementary Data – Phenoarrays.

Biolog Plate	Well position		Biolog compound in well			*S.aureus NCTC8325* Biolog end point reading at 47 hour (background subtracted)			
ID	row	column	ID	mode	other info	0.5 x MIC MGB-BP3	0.25 x MIC MGB-BP3	0.125 x MIC MGB-BP3	ctrl (DMSO)
PM1	B	12	L-glutamic acid	C-source	amino acid	0	182	171	203
PM2A	H	1	L-ornithine	C-source	amino acid	175	203	201	202
PM2A	B	12	3-0-b-D-galactopyranosyl-D-arabinose	C-source	carbohydrate	19	199	191	199
PM1	G	12	L-malic acid	C-source	carboxylic acid	1	179	170	198
PM1	A	3	N-acetyl-D-Glucosamine	C-source	carbohydrate	0	144	146	197
PM1	G	1	glycyl-L-glutamic acid	C-source	amino acid	0	170	177	197
PM1	A	11	D-mannose	C-source	carbohydrate	0	122	137	195
PM1	C	4	D-ribose	C-source	carbohydrate	115	177	157	191
PM1	D	12	uridine	C-source	carbohydrate	100	178	171	191
PM1	B	7	D,L-a-glycerol phosphate	C-source	carbohydrate	77	170	161	190
PM1	A	10	D-trehalose	C-source	carbohydrate	68	114	144	188
PM1	C	12	**thymidine**	C-source	carbohydrate	0	69	142	188
PM2A	G	4	**L-arginine**	C-source	amino acid	81	133	172	187
PM1	A	5	succinic acid	C-source	carboxylic acid	0	165	161	186
PM1	B	11	D-mannitol	C-source	carbohydrate	0	148	136	186
PM1	C	7	D-fructose	C-source	carbohydrate	0	135	135	183
PM1	E	1	**L-glutamine**	C-source	amino acid	0	86	125	183
PM1	D	11	sucrose	C-source	carbohydrate	30	135	134	182
PM1	E	4	D-fructose-6-phosphate	C-source	carbohydrate	0	163	156	181
PM1	D	6	a-ketoglutaric acid	C-source	carboxylic acid	109	162	152	180
PM2A	B	2	N-acetyl-neuraminic acid	C-source	carboxylic acid	120	162	161	178
PM1	B	6	D-gluconic acid	C-source	carboxylic acid	0	141	145	177
PM1	D	9	a-D-lactose	C-source	carbohydrate	7	126	116	177
PM1	C	1	D-glucose-6-phosphate	C-source	carbohydrate	48	164	125	176
PM2A	H	9	dihydroxyacetone	C-source	alcohol	158	178	156	176
PM1	A	8	**L-proline**	C-source	amino acid	74	149	136	172
PM1	C	3	D,L-malic acid	C-source	carboxylic acid	0	125	125	172
PM1	C	10	maltose	C-source	carbohydrate	30	124	123	172
PM2A	G	6	L-histidine	C-source	amino acid	116	159	151	169
PM1	E	10	maltotriose	C-source	carbohydrate	52	110	120	168
PM1	C	9	a-D-glucose	C-source	carbohydrate	21	127	134	167
PM2A	G	5	**glycine**	C-source	amino acid	0	123	113	163
PM1	G	8	N-acetyl-D-mannosamine	C-source	carbohydrate	0	130	124	162
PM1	B	8	D-xylose	C-source	carbohydrate	90	124	107	159
PM1	F	5	fumaric acid	C-source	carboxylic acid	35	101	126	159
PM1	G	4	**L-threonine**	C-source	amino acid	14	111	90	155
PM1	A	6	D-galactose	C-source	carbohydrate	111	148	162	154
PM1	F	12	inosine	C-source	carbohydrate	62	165	155	154
PM2A	E	12	5-keto-D-gluconic acid	C-source	carboxylic acid	134	153	137	153
PM1	D	10	lactulose	C-source	carbohydrate	2	109	137	149
PM1	E	8	b-methyl-D-glucoside	C-source	carbohydrate	16	110	112	148
PM1	A	2	L-Arabinose	C-source	carbohydrate	98	128	111	139
PM1	B	3	glycerol	C-source	carbohydrate	56	133	104	139
PM1	B	10	formic acid	C-source	carboxylic acid	0	107	98	139
PM2A	D	7	turanose	C-source	carbohydrate	68	130	123	134
PM1	B	9	L-lactic acid	C-source	carboxylic acid	10	108	102	128
PM2A	C	5	maltitol	C-source	carbohydrate	83	157	164	124
PM2A	B	5	D-arabinose	C-source	carbohydrate	109	112	101	119
PM2A	B	9	2-deoxy-D-ribose	C-source	carbohydrate	101	119	115	119
PM2A	E	5	D-glucosamine	C-source	carbohydrate	104	116	116	113
PM1	G	6	L-alanyl-glycine	C-source	amino acid	13	134	68	98
PM1	H	6	L-lyxose	C-source	carbohydrate	110	157	132	95
PM1	H	8	pyruvic acid	C-source	carboxylic acid	66	113	85	93
PM2A	A	6	dextrin	C-source	polymer	63	86	85	89
PM2A	C	12	palatinose	C-source	carbohydrate	56	59	56	74
PM2A	F	9	sorbic acid	C-source	carboxylic acid	33	88	53	74
PM2A	B	8	arbutin	C-source	carbohydrate	33	47	49	72
PM1	G	7	acetoacetic acid	C-source	carboxylic acid	0	40	18	69
PM1	F	1	glycyl-L-aspartic acid	C-source	amino acid	0	6	0	59
PM2A	F	5	oxalomalic acid	C-source	carboxylic acid	43	52	39	58
PM1	F	2	citric acid	C-source	carboxylic acid	0	0	30	57
PM1	H	1	glycyl-L-proline	C-source	amino acid	56	130	83	56
PM1	G	5	L-alanine	C-source	amino acid	0	40	0	54

Biolog data analysis (Biolog projects L14 and L15)	
after background subtraction:	
Biolog end point reading	**Interpretation**
0-49	no growth
50-122	reduced growth
123-154	growth
>155	full growth
	traffic light between 0.25 x MIC, 0.125 x MIC and ctrl

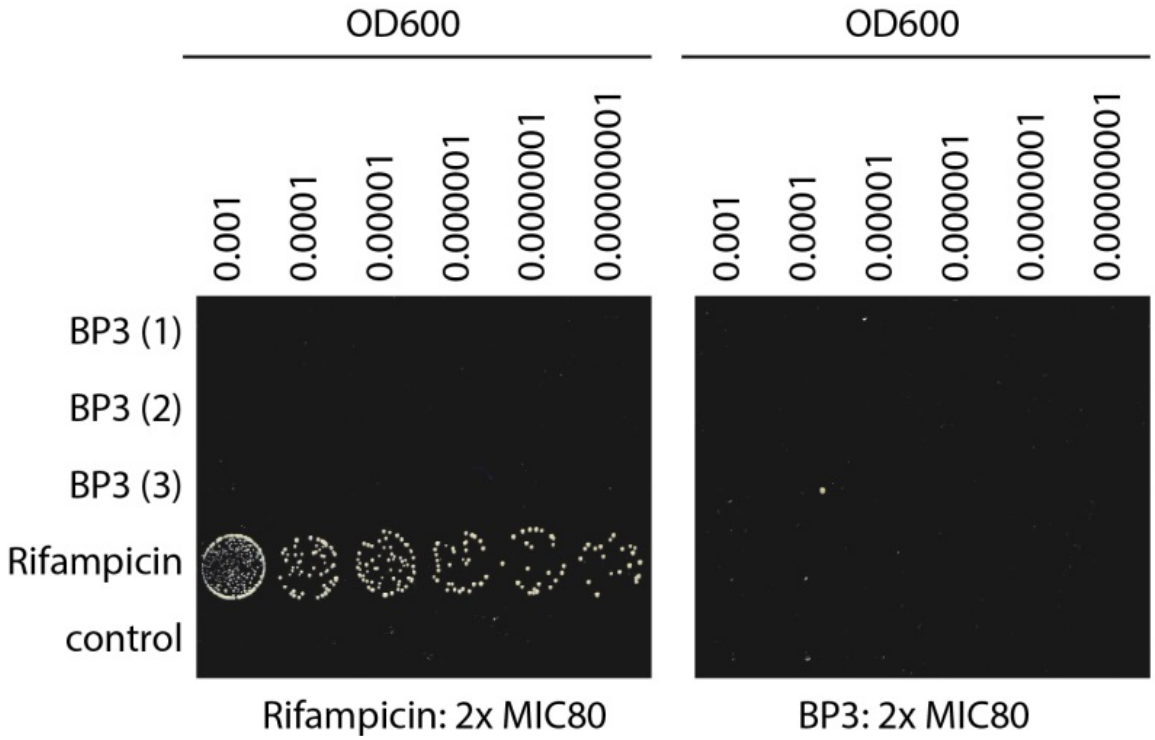
Note: traffic light data analysis does not take into account the respiration curve shape, only end point (at 47 hour)

Note: single Biolog plates run (n=1)

Note: above tresholds for traffic light analysis validated via visual inspection of PM1 plate's respiration curves when background was not subtracted

additionally highlighted from visual inspection of respiration curves when background was not subtracted

Supplementary Data – Resistance. Assessment of S. aureus resistance to MGB-BP-3 following serial passaging in LB containing either MGB-BP-3 (3 independent populations at up to 0.5x MIC80), rifampicin (one population at up to 0.4x MIC80), or no antibiotic (control) for 80 generations. Cultures at corresponding OD600 were spotted on agar plates containing either rifampicin or MGB-BP-3 at 2x MIC80 concentrations.



Supplementary Table 1: Primers used in qRT-PCR expression studies and Melt off analysis

Locus tag	**Gene**	**Name**	**Sequence (5'-3')**
qRT-PCR:			
SAOUHSC_00579	*mvaK2*	Saur_00579f	TTAATCAAAAACTGGCCTGGAT
SAOUHSC_00579	*mvaK2*	Saur_00579r	TTCGCTAACAAAGTGTGGTGAT
SAOUHSC_00795	*gapA*	Saur_00795f	GGCAGAAAACATCATCCCTAAC
SAOUHSC_00795	*gapA*	Saur_00795r	TGAAGCATTTTTCATAGCTTCG
SAOUHSC_00898	*argH*	Saur_00898f	CTGGCTCATCTATTATGCCACA
SAOUHSC_00898	*argH*	Saur_00898r	GGACAGCATCGAATAAACCTTC
SAOUHSC_01171	*pyrF*	Saur_01171f	TTAGATGGCGTTGTTTGTTCAC
SAOUHSC_01171	*pyrF*	Saur_01171r	GGTCATTTTGAGATGCACCTTT
SAOUHSC_01490	*hup*	Saur_01490f	TTGCAGAGCAAGCTGATTTAAC
SAOUHSC_01490	*hup*	Saur_01490r	ACCTCAAAGTTACCGAAACCAA
SAOUHSC_01670	*cdd*	Saur_01670f	CGTAGATGCAGATAAACCGTCA
SAOUHSC_01670	*cdd*	Saur_01670r	CTGCGACTGTCATCATAACCAT
SAOUHSC_01801	*citC*	Saur_01801f	ACTTGGCAACAATATGACCAAA
SAOUHSC_01801	*citC*	Saur_01801r	GCTCAGCTGGACGAGTTAAAAT

Melt off analysis:

SAOUHSC_00006	*gyrA*	SAOUHSC_00006for	AGAGCCGTCAGTCTTACCTG
SAOUHSC_00006	*gyrA*	SAOUHSC_00006rev	ACCTACCGCGATACCTGATG
SAOUHSC_01146	*mraY*	SAOUHSC_01146for	ATTTGAAAAATTGATAATATATTAGTG
SAOUHSC_01146	*mraY*	SAOUHSC_01146rev	CGCATATACAAAAATCATAACTATCT
SAOUHSC_01470	*dnaD*	SAOUHSC_01470for	AGCATTTTAGTCGAAAATTAAAGAA
SAOUHSC_01470	*dnaD*	SAOUHSC_01470rev	GATATTTATCCATGTTCGTGC

Supplementary Table 2. List of genes differentially expressed by a factor of ≥ 2 or ≤ -2 in MGB-BP-3 treated *S. aureus NCTC8325* compared to non-treated controls after 10 min of exposure.

Locus tag	Gene	Product	Fold change
Downregulated			
Amino acid biosynthesis			
SAOUHSC_00413	*mpsB*	hypothetical protein	-3
SAOUHSC_01055		inositol monophosphatase family protein	-2
SAOUHSC_01597	*proC*	pyrroline-5-carboxylate reductase	-4
SAOUHSC_01617	*argR*	arginine repressor	-4
SAOUHSC_01998		hypothetical protein	-3
Biosynthesis of cofactors, prosthetic groups, and carriers			
SAOUHSC_00225	*ispD*	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	-2
SAOUHSC_00723	*pabB*	hypothetical protein	-4
SAOUHSC_00724		chorismate binding protein	-5
SAOUHSC_00847	*sufC*	ABC transporter ATP-binding protein	-3
SAOUHSC_00848	*sufD*	hypothetical protein	-2
SAOUHSC_00980	*menA*	1,4-dihydroxy-2-naphthoate octaprenyltransferase	-4
SAOUHSC_01178	*coaBC*	bifunctional phosphopantothenoylcysteine decarboxylase/phosphopantothenate--cysteine ligase	-4
SAOUHSC_01190	*thiN*	hypothetical protein	-4
SAOUHSC_01356	*glcT*	transcription antiterminator	-8
SAOUHSC_01486	*hepT*	heptaprenyl diphosphate syntase component II	-4
SAOUHSC_01487	*ubiE*	ubiquinone/menaquinone biosynthesis methyltransferase	-3
SAOUHSC_01776	*hemA*	glutamyl-tRNA reductase	-4
SAOUHSC_01795	*coaE*	dephospho-CoA kinase	-2
SAOUHSC_01882		hypothetical protein	-4
SAOUHSC_01915	*menC*	hypothetical protein	-3
SAOUHSC_01916	*menE*	2-succinylbenzoate-CoA ligase	-6
SAOUHSC_02132	*nadE*	NAD synthetase	-3
SAOUHSC_02133		nicotinate phosphoribosyltransferase	-3
Cell envelope			
SAOUHSC_00279		hypothetical protein	-5

SAOUHSC_00471	*glmU*	bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase	-3
SAOUHSC_00535		hypothetical protein	-3
SAOUHSC_00640	*tagA*	teichoic acid biosynthesis protein	-2
SAOUHSC_00643	*tagB*	teichoic acid biosynthesis protein TagB	-5
SAOUHSC_00752	*murB*	UDP-N-acetylenolpyruvoylglucosamine reductase	-3
SAOUHSC_00953	*ugtP*	diacylglycerol glucosyltransferase	-5
SAOUHSC_01051		hypothetical protein	-4
SAOUHSC_01063		hypothetical protein	-5
SAOUHSC_01145	*pbp1*	penicillin-binding protein 1	-4
SAOUHSC_01146	*mraY*	phospho-N-acetylmuramoyl-pentapeptide- transferase	-4
SAOUHSC_01147	*murD*	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	-2
SAOUHSC_01758	*mreD*	hypothetical protein	-3
SAOUHSC_01759	*mreC*	rod shape-determining protein MreC	-3
SAOUHSC_01812		hypothetical protein	-3
SAOUHSC_02305	*alr*	alanine racemase	-3
SAOUHSC_02319		hypothetical protein	-4

Cellular processes

SAOUHSC_00024	*walJ*	hypothetical protein	-3
SAOUHSC_00185	*hptS*	hypothetical protein	-5
SAOUHSC_00299		hypothetical protein	-4
SAOUHSC_00479		hypothetical protein	-4
SAOUHSC_00685		hypothetical protein	-4
SAOUHSC_00789	*whiA*	hypothetical protein	-2
SAOUHSC_00819	*cspC*	hypothetical protein	-4
SAOUHSC_00948		hypothetical protein	-4
SAOUHSC_01039		hypothetical protein	-9
SAOUHSC_01063		hypothetical protein	-5
SAOUHSC_01142	*mraZ*	cell division protein MraZ	-8
SAOUHSC_01144	*ftsL*	cell division protein	-9
SAOUHSC_01145	*pbp1*	penicillin-binding protein 1	-4
SAOUHSC_01213		hypothetical protein	-4
SAOUHSC_01317		hypothetical protein	-3
SAOUHSC_01382		hypothetical protein	-3
SAOUHSC_01407		hypothetical protein	-4
SAOUHSC_01431	*msrB*	methionine sulfoxide reductase B	-3
SAOUHSC_01432	*msrA1*	methionine sulfoxide reductase A	-3
SAOUHSC_01827	*ezrA*	septation ring formation regulator EzrA	-3
SAOUHSC_01871		polysaccharide biosynthesis protein	-3
SAOUHSC_01999		hypothetical protein	-3
SAOUHSC_02098	*vraR*	DNA-binding response regulator VraR	-4
SAOUHSC_02121		hypothetical protein	-3
SAOUHSC_02319		hypothetical protein	-4
SAOUHSC_02591		hypothetical protein	-6

SAOUHSC_02811	*relP*	hypothetical protein	-2
Central intermediary metabolism			
SAOUHSC_00471	*glmU*	bifunctional N-acetylglucosamine-1-phosphate uridyltransferase/glucosamine-1-phosphate acetyltransferase	-3
SAOUHSC_00552	*nagB*	hypothetical protein	-3
SAOUHSC_01650		5-formyltetrahydrofolate cyclo-ligase	-3
SAOUHSC_01987		hypothetical protein	-2
SAOUHSC_02011	*recX*	recombination regulator RecX	-4
SAOUHSC_02142	*aldH*	aldehyde dehydrogenase	-3
SAOUHSC_02259		hypothetical protein	-2
SAOUHSC_02793		hypothetical protein	-3
DNA metabolism			
SAOUHSC_00162	*hsdR*	HsdR family type I site-specific deoxyribonuclease	-3
SAOUHSC_00477	*mfd*	transcription-repair coupling factor	-3
SAOUHSC_00819	*cspC*	hypothetical protein	-4
SAOUHSC_01039		hypothetical protein	-9
SAOUHSC_01179	*priA*	primosomal protein N	-2
SAOUHSC_01333	*lexA*	LexA repressor	-2
SAOUHSC_01336		hypothetical protein	-3
SAOUHSC_01454		hypothetical protein	-5
SAOUHSC_01469	*nth*	endonuclease III	-3
SAOUHSC_01470	*dnaD*	hypothetical protein	-5
SAOUHSC_01472	*dinG*	DnaQ family exonuclease/DinG family helicase	-2
SAOUHSC_01615	*recN*	DNA repair protein RecN	-3
SAOUHSC_01667	*recO*	hypothetical protein	-3
SAOUHSC_01673	*phoH*	hypothetical protein	-6
SAOUHSC_01796	*mutM*	formamidopyrimidine-DNA glycosylase	-3
SAOUHSC_01797	*polA*	DNA polymerase I	-5
SAOUHSC_01811	*dnaE*	DNA polymerase III subunit alpha superfamily protein	-3
SAOUHSC_01827	*ezrA*	septation ring formation regulator EzrA	-3
Energy metabolism			
SAOUHSC_00100	*deoC1*	2-deoxyribose-5-phosphate aldolase	-4
SAOUHSC_00226	*tarJ*	hypothetical protein	-3
SAOUHSC_00412	*mpsA*	NADH dehydrogenase subunit 5	-6
SAOUHSC_00616		hypothetical protein	-3
SAOUHSC_00756		hypothetical protein	-3
SAOUHSC_01189	*cfxE*	ribulose-phosphate 3-epimerase	-5
SAOUHSC_01599	*zwf*	glucose-6-phosphate 1-dehydrogenase	-4
SAOUHSC_01665		CBS domain-containing protein	-3
SAOUHSC_01801	*citC*	isocitrate dehydrogenase	-3
SAOUHSC_01867	*dat*	D-alanine aminotransferase	-4
SAOUHSC_01901	*tal*	putative translaldolase	-3

SAOUHSC_01912		hypothetical protein	-4
SAOUHSC_02550	*fdhD*	formate dehydrogenase accessory protein	-3
SAOUHSC_02808	*gntK*	gluconate kinase	-3
SAOUHSC_02845		hypothetical protein	-6
Fatty acid and phospholipid metabolism			
SAOUHSC_01350	*plsY*	hypothetical protein	-2
SAOUHSC_02306	*acpS*	4'-phosphopantetheinyl transferase	-3
Mobile and extrachromosomal element functions			
SAOUHSC_01374	*femB*	methicillin resistance factor	-3
SAOUHSC_01464		hypothetical protein	-4
SAOUHSC_01470	*dnaD*	hypothetical protein	-5
Protein fate			
SAOUHSC_00547		hypothetical protein	-3
SAOUHSC_00639		hypothetical protein	-4
SAOUHSC_00963	*lplA1*	lipoyltransferase and lipoate-protein ligase	-3
SAOUHSC_00973	*tarM*	hypothetical protein	-4
SAOUHSC_01038	*def*	peptide deformylase	-3
SAOUHSC_01182	*def2*	peptide deformylase	-3
SAOUHSC_01406	*acyP*	acylphosphatase	-4
SAOUHSC_01431	*msrB*	methionine sulfoxide reductase B	-3
SAOUHSC_01432	*msrA1*	methionine sulfoxide reductase A	-3
SAOUHSC_01626	*pepQ2*	proline dipeptidase	-2
SAOUHSC_01629	*lipM*	hypothetical protein	-4
SAOUHSC_01838	*htrA1*	hypothetical protein	-4
SAOUHSC_02013		hypothetical protein	-3
SAOUHSC_02102	*map*	methionine aminopeptidase	-6
SAOUHSC_02472		hypothetical protein	-4
SAOUHSC_02984		accessory Sec system glycosyltransferase GtfA	-3
Protein synthesis			
SAOUHSC_00475	*pth*	peptidyl-tRNA hydrolase	-5
SAOUHSC_00605		hypothetical protein	-3
SAOUHSC_00663		hypothetical protein	-2
SAOUHSC_00933	*trpS*	tryptophanyl-tRNA synthetase	-3
SAOUHSC_00979		hypothetical protein	-12
SAOUHSC_01091		hypothetical protein	-2
SAOUHSC_01143	*mraW*	16S rRNA (cytosine(1402)-N(4))-methyltransferase	-9
SAOUHSC_01183	*fmt*	methionyl-tRNA formyltransferase	-2
SAOUHSC_01188	*rsgA*	hypothetical protein	-6
SAOUHSC_01214	*rbgA*	ribosomal biogenesis GTPase	-3
SAOUHSC_01455		hypothetical protein	-5
SAOUHSC_01668	*era*	GTP-binding protein Era	-4
SAOUHSC_01672	*ybeY*	hypothetical protein	-5

SAOUHSC_01865	*trmB*	tRNA (guanine-N(7)-)-methyltransferase	-3
SAOUHSC_01870		16S rRNA pseudouridine(516) synthase	-3
SAOUHSC_02297		S1 RNA-binding domain-containing protein	-2
SAOUHSC_02480	*truA*	tRNA pseudouridine synthase A	-2
SAOUHSC_02519		hypothetical protein	-3
SAOUHSC_02651		hypothetical protein	-3

Purines, pyrimidines, nucleosides, and nucleotides

SAOUHSC_00100	*deoC1*	2-deoxyribose-5-phosphate aldolase	-4
SAOUHSC_00101	*deoB*	phosphopentomutase	-3
SAOUHSC_00741	*nrdI*	ribonucleotide reductase stimulatory protein	-4
SAOUHSC_00743	*nrdF*	ribonucleotide-diphosphate reductase subunit beta	-4
SAOUHSC_01435	*thyA*	thymidylate synthase	-3
SAOUHSC_01670	*cdd*	cytidine deaminase	-4

Regulatory functions

SAOUHSC_00020	*walR*	two-component response regulator	-4
SAOUHSC_00070	*sarS*	accessory regulator-like protein	-4
SAOUHSC_00231	*lytR*	two-component response regulator	-4
SAOUHSC_00242	*rbsR*	hypothetical protein	-2
SAOUHSC_00665	*graR*	hypothetical protein	-3
SAOUHSC_00674	*sarX*	hypothetical protein	-4
SAOUHSC_00675		hypothetical protein	-3
SAOUHSC_00679	*ccpE*	hypothetical protein	-5
SAOUHSC_00685		hypothetical protein	-4
SAOUHSC_01142	*mraZ*	cell division protein MraZ	-8
SAOUHSC_01285	*glnR*	glutamine synthetase repressor	-3
SAOUHSC_01333	*lexA*	LexA repressor	-2
SAOUHSC_01361	*msrR*	transcriptional regulator	-4
SAOUHSC_01464		hypothetical protein	-4
SAOUHSC_01586	*srrA*	DNA-binding response regulator	-3
SAOUHSC_01617	*argR*	arginine repressor	-4
SAOUHSC_01685	*hrcA*	heat-inducible transcription repressor HrcA	-4
SAOUHSC_01793	*nrdR*	transcriptional regulator NrdR	-2
SAOUHSC_01800	*phoP*	alkaline phosphatase synthesis transcriptional regulatory protein	-5
SAOUHSC_02014		hypothetical protein	-3
SAOUHSC_02300	*rsbV*	STAS domain-containing protein	-6
SAOUHSC_02456	*lacR*	lactose phosphotransferase system repressor	-5
SAOUHSC_02583		transcriptional regulator	-2
SAOUHSC_02635	*tcaA*	hypothetical protein	-2
SAOUHSC_02956	*nsaR*	nisin susceptibility-associated DNA-binding response regulator	-3

Signal transduction

SAOUHSC_00020	*walR*	two-component response regulator	-4
SAOUHSC_00021	*walK*	sensory box histidine kinase VicK	-4
SAOUHSC_00230	*lytS*	two-component sensor histidine kinase	-4
SAOUHSC_00313		hypothetical protein	-2
SAOUHSC_00665	*graR*	hypothetical protein	-3
SAOUHSC_00666	*graS*	hypothetical protein	-4
SAOUHSC_01586	*srrA*	DNA-binding response regulator	-3
SAOUHSC_01799	*phoR*	histidine kinase	-4
SAOUHSC_01800	*phoP*	alkaline phosphatase synthesis transcriptional regulatory protein	-5
SAOUHSC_02400	*mtlF*	PTS system mannitol-specific protein	-6
SAOUHSC_02955	*nsaS*	nisin susceptibility-associated sensor histidine kinase	-2
SAOUHSC_02956	*nsaR*	nisin susceptibility-associated DNA-binding response regulator	-3
SAOUHSC_02974		hypothetical protein	-3

Transcription

SAOUHSC_01035	*rnjA*	hypothetical protein	-3
SAOUHSC_01252	*rnjB*	hypothetical protein	-3

Transport and binding proteins

SAOUHSC_00060		hypothetical protein	-3
SAOUHSC_00067	*lctP1*	L-lactate permease	-3
SAOUHSC_00114	*capA*	capsular polysaccharide biosynthesis protein	-5
SAOUHSC_00151	*brnQ1*	branched-chain amino acid transport system II carrier protein	-5
SAOUHSC_00175	*malK*	multiple sugar-binding transport ATP-binding protein	-5
SAOUHSC_00186	*hptA*	lipoprotein	-3
SAOUHSC_00313		hypothetical protein	-2
SAOUHSC_00556	*proP*	proline/betaine transporter	-3
SAOUHSC_00633		hypothetical protein	-3
SAOUHSC_00641	*tagH*	teichoic acids export protein ATP-binding subunit	-2
SAOUHSC_00681		major facilitator superfamily protein superfamily	-3
SAOUHSC_00731	*opuBA*	ABC transporter	-4
SAOUHSC_00740		hypothetical protein	-8
SAOUHSC_00787		hypothetical protein	-5
SAOUHSC_00885	*mnhE*	monovalent cation/H+ antiporter subunit E	-3
SAOUHSC_00886	*mnhD*	monovalent cation/H+ antiporter subunit D	-3
SAOUHSC_00887	*mnhC*	monovalent cation/H+ antiporter subunit C	-3
SAOUHSC_00888	*mnhB*	monovalent cation/H+ antiporter subunit B	-4

SAOUHSC_00889	*mnhA*	monovalent cation/H+ antiporter subunit A	-5
SAOUHSC_00923	*opp-3B*	hypothetical protein	-4
SAOUHSC_00924	*opp-3C*	hypothetical protein	-4
SAOUHSC_00925	*opp-3D*	hypothetical protein	-3
SAOUHSC_01326		hypothetical protein	-2
SAOUHSC_01505		hypothetical protein	-3
SAOUHSC_01803	*aapA*	hypothetical protein	-5
SAOUHSC_01967		ABC transporter ATP-binding protein	-3
SAOUHSC_02006		hypothetical protein	-4
SAOUHSC_02009		hypothetical protein	-6
SAOUHSC_02247		cation transport protein	-4
SAOUHSC_02400	*mtlF*	PTS system mannitol-specific protein	-6
SAOUHSC_02482	*cbiO_1*	cobalt transporter ATP-binding subunit	-3
SAOUHSC_02483	*cbiO_2*	cobalt transporter ATP-binding subunit	-4
SAOUHSC_02597	*glvC*	PTS system transporter	-4
SAOUHSC_02601		Na+/H+ antiporter	-2
SAOUHSC_02729		amino acid ABC transporter-like protein	-7
SAOUHSC_02797		hypothetical protein	-5
SAOUHSC_02806	*gntP*	gluconate permease	-3
SAOUHSC_02815		hypothetical protein	-5
SAOUHSC_02974		hypothetical protein	-3

Unknown TIGRFAM main role

SAOUHSC_00014		hypothetical protein	-3
SAOUHSC_00022	*walH*	hypothetical protein	-3
SAOUHSC_00023	*walI*	hypothetical protein	-2
SAOUHSC_00056		hypothetical protein	-3
SAOUHSC_00125	*capL*	cap5L protein/glycosyltransferase	-4
SAOUHSC_00130	*isdI*	heme-degrading monooxygenase IsdI	-3
SAOUHSC_00163		hypothetical protein	-5
SAOUHSC_00164		hypothetical protein	-5
SAOUHSC_00166		hypothetical protein	-3
SAOUHSC_00176	*malE*	extracellular solute-binding protein	-4
SAOUHSC_00178		maltose ABC transporter permease	-3
SAOUHSC_00227	*tarL*	hypothetical protein	-2
SAOUHSC_00232	*lrgA*	murein hydrolase regulator LrgA	-5
SAOUHSC_00256		hypothetical protein	-3
SAOUHSC_00322		hypothetical protein	-3
SAOUHSC_00324		50S ribosomal protein L7 serine acetyltransferase	-4
SAOUHSC_00342		ParB family chromosome partioning protein	-4
SAOUHSC_00370		hypothetical protein	-3
SAOUHSC_00382		hypothetical protein	-4
SAOUHSC_00450		Orn/Lys/Arg decarboxylase	-3
SAOUHSC_00585		hypothetical protein	-5

SAOUHSC_00586		hypothetical protein	-6
SAOUHSC_00587		hypothetical protein	-6
SAOUHSC_00588		hypothetical protein	-6
SAOUHSC_00593		unknown	-78
SAOUHSC_00613		iron compound ABC transporter substrate-binding protein	-3
SAOUHSC_00614		hypothetical protein	-3
SAOUHSC_00615		haloacid dehalogenase-like hydrolase	-4
SAOUHSC_00664	*graX*	hypothetical protein	-3
SAOUHSC_00673		hypothetical protein	-5
SAOUHSC_00678		hypothetical protein	-6
SAOUHSC_00682		hypothetical protein	-3
SAOUHSC_00683		hypothetical protein	-6
SAOUHSC_00686		hypothetical protein	-5
SAOUHSC_00687		hypothetical protein	-4
SAOUHSC_00692		hypothetical protein	-3
SAOUHSC_00693		hypothetical protein	-3
SAOUHSC_00712		hypothetical protein	-3
SAOUHSC_00722	*pabA*	para-aminobenzoate synthase glutamine amidotransferase component II	-6
SAOUHSC_00726		hypothetical protein	-4
SAOUHSC_00727		hypothetical protein	-3
SAOUHSC_00732	*opuBB*	amino acid ABC transporter permease	-2
SAOUHSC_00736		hypothetical protein	-3
SAOUHSC_00742	*nrdE*	ribonucleotide-diphosphate reductase subunit alpha	-3
SAOUHSC_00751		hypothetical protein	-3
SAOUHSC_00774		hypothetical protein	-3
SAOUHSC_00783		hypothetical protein	-3
SAOUHSC_00784		hypothetical protein	-3
SAOUHSC_00788		hypothetical protein	-4
SAOUHSC_00800		hypothetical protein	-5
SAOUHSC_00824		hypothetical protein	-11
SAOUHSC_00830		hypothetical protein	-4
SAOUHSC_00854		hypothetical protein	-5
SAOUHSC_00855		hypothetical protein	-5
SAOUHSC_00860		5-nucleotidase family protein	-2
SAOUHSC_00864		hypothetical protein	-3
SAOUHSC_00865		hypothetical protein	-3
SAOUHSC_00875		hypothetical protein	-4
SAOUHSC_00890	*kapB*	hypothetical protein	-5
SAOUHSC_00906		hypothetical protein	-3
SAOUHSC_00938	*yjbH*	hypothetical protein	-7
SAOUHSC_00939		hypothetical protein	-6
SAOUHSC_00940		hypothetical protein	-3
SAOUHSC_00952	*ltaA*	hypothetical protein	-4
SAOUHSC_00962		hypothetical protein	-4
SAOUHSC_00974		hypothetical protein	-5
SAOUHSC_01025		hypothetical protein	-6
SAOUHSC_01036	*rpoY*	hypothetical protein	-3

SAOUHSC_01050		hypothetical protein	-3
SAOUHSC_01082	*isdC*	hypothetical protein	-3
SAOUHSC_01119		hypothetical protein	-3
SAOUHSC_01120		hypothetical protein	-2
SAOUHSC_01152		hypothetical protein	-3
SAOUHSC_01265		hypothetical protein	-6
SAOUHSC_01266		hypothetical protein	-5
SAOUHSC_01267		2-oxoglutarate ferredoxin oxidoreductase subunit beta	-3
SAOUHSC_01354	*alsT*	sodium:alanine symporter family protein	-2
SAOUHSC_01359	*fmtC*	hypothetical protein	-3
SAOUHSC_01391	*cvfB*	hypothetical protein	-2
SAOUHSC_01405		hypothetical protein	-5
SAOUHSC_01408		hypothetical protein	-4
SAOUHSC_01414		hypothetical protein	-2
SAOUHSC_01419	*arlS*	hypothetical protein	-3
SAOUHSC_01429		hypothetical protein	-2
SAOUHSC_01433	*fakB2*	hypothetical protein	-3
SAOUHSC_01434	*dfrA*	dihydrofolate reductase	-3
SAOUHSC_01463		hypothetical protein	-3
SAOUHSC_01474	*papS*	tRNA CCA-pyrophosphorylase	-3
SAOUHSC_01488		hypothetical protein	-4
SAOUHSC_01584		hypothetical protein	-4
SAOUHSC_01596		hypothetical protein	-5
SAOUHSC_01600		hypothetical protein	-4
SAOUHSC_01606		peptidase T	-2
SAOUHSC_01614	*lpdA*	dihydrolipoamide dehydrogenase	-2
SAOUHSC_01627		hypothetical protein	-5
SAOUHSC_01645		hypothetical protein	-2
SAOUHSC_01646	*glk*	glucokinase	-2
SAOUHSC_01648		hypothetical protein	-2
SAOUHSC_01664		hypothetical protein	-2
SAOUHSC_01671	*dgkA*	diacylglycerol kinase	-5
SAOUHSC_01684	*grpE*	heat shock protein GrpE	-3
SAOUHSC_01706		hypothetical protein	-8
SAOUHSC_01798		hypothetical protein	-4
SAOUHSC_01802	*citZ*	hypothetical protein	-4
SAOUHSC_01847	*acuA*	hypothetical protein	-4
SAOUHSC_01849	*acuC*	acetoin utilization protein AcuC	-4
SAOUHSC_01868		dipeptidase PepV	-4
SAOUHSC_01895		hypothetical protein	-4
SAOUHSC_01896		hypothetical protein	-7
SAOUHSC_01908		hypothetical protein	-4
SAOUHSC_01914		hypothetical protein	-3
SAOUHSC_01957		hypothetical protein	-5
SAOUHSC_01958		hypothetical protein	-3
SAOUHSC_01966		hypothetical protein	-3
SAOUHSC_01986		hypothetical protein	-3
SAOUHSC_01997	*perR*	ferric uptake regulator-like protein	-5
SAOUHSC_02004		hypothetical protein	-3

SAOUHSC_02007		hypothetical protein	-5
SAOUHSC_02008		hypothetical protein	-6
SAOUHSC_02010		hypothetical protein	-5
SAOUHSC_02087		hypothetical protein	-3
SAOUHSC_02093		hypothetical protein	-2
SAOUHSC_02100	*vraT*	hypothetical protein	-8
SAOUHSC_02101	*vraU*	hypothetical protein	-6
SAOUHSC_02103		hypothetical protein	-3
SAOUHSC_02111	*dinP*	DNA polymerase IV	-4
SAOUHSC_02114		lipid kinase	-4
SAOUHSC_02131		hypothetical protein	-2
SAOUHSC_02134		nitric oxide synthase oxygenase subunit	-3
SAOUHSC_02135	*pheA*	hypothetical protein	-4
SAOUHSC_02139		pyrazinamidase/nicotinamidase	-3
SAOUHSC_02146		hypothetical protein	-3
SAOUHSC_02147		hypothetical protein	-6
SAOUHSC_02148		hypothetical protein	-6
SAOUHSC_02149		hypothetical protein	-7
SAOUHSC_02256		hypothetical protein	-5
SAOUHSC_02257	*sdrH*	hypothetical protein	-6
SAOUHSC_02273	*rex*	redox-sensing transcriptional repressor Rex	-3
SAOUHSC_02274	*vga*	ABC transporter ATP-binding protein	-3
SAOUHSC_02298	*sigB*	RNA polymerase sigma factor SigB	-6
SAOUHSC_02299	*rsbW*	serine-protein kinase RsbW	-4
SAOUHSC_02301	*rsbU*	putative sigmaB regulation protein	-3
SAOUHSC_02302		hypothetical protein	-3
SAOUHSC_02303	*mazF*	hypothetical protein	-4
SAOUHSC_02304	*mazE*	hypothetical protein	-3
SAOUHSC_02307		hypothetical protein	-3
SAOUHSC_02308		hypothetical protein	-6
SAOUHSC_02309		hypothetical protein	-9
SAOUHSC_02335	*ywpF*	hypothetical protein	-7
SAOUHSC_02367		hypothetical protein	-4
SAOUHSC_02383		hypothetical protein	-3
SAOUHSC_02390		lytic regulatory protein	-7
SAOUHSC_02396		hypothetical protein	-2
SAOUHSC_02428	*htsB*	hypothetical protein	-3
SAOUHSC_02430	*htsA*	ABC transporter substrate-binding protein	-3
SAOUHSC_02448		hypothetical protein	-7
SAOUHSC_02464		hypothetical protein	-4
SAOUHSC_02465		hypothetical protein	-4
SAOUHSC_02471		hypothetical protein	-5
SAOUHSC_02473		hypothetical protein	-8
SAOUHSC_02474		hypothetical protein	-5
SAOUHSC_02556		hypothetical protein	-4
SAOUHSC_02567		hypothetical protein	-3
SAOUHSC_02574		hypothetical protein	-3
SAOUHSC_02582	*fdhA*	formate dehydrogenase subunit alpha	-3

SAOUHSC_02592		hypothetical protein	-5
SAOUHSC_02611	*lyrA*	hypothetical protein	-3
SAOUHSC_02613		hypothetical protein	-2
SAOUHSC_02631		hypothetical protein	-4
SAOUHSC_02633		hypothetical protein	-4
SAOUHSC_02638		hypothetical protein	-3
SAOUHSC_02650		hypothetical protein	-2
SAOUHSC_02663		hypothetical protein	-4
SAOUHSC_02669	*sarZ*	hypothetical protein	-3
SAOUHSC_02686		hypothetical protein	-3
SAOUHSC_02694	*dsbA*	hypothetical protein	-4
SAOUHSC_02725		hypothetical protein	-4
SAOUHSC_02727		hypothetical protein	-15
SAOUHSC_02736		hypothetical protein	-3
SAOUHSC_02787		hypothetical protein	-3
SAOUHSC_02789		hypothetical protein	-6
SAOUHSC_02790		hypothetical protein	-3
SAOUHSC_02816		hypothetical protein	-4
SAOUHSC_02846		hypothetical protein	-10
SAOUHSC_02880	*crtQ*	hypothetical protein	-2
SAOUHSC_02910		hypothetical protein	-3
SAOUHSC_02929		acetyl-CoA synthetase	-5
SAOUHSC_02931		hypothetical protein	-22
SAOUHSC_02947	*cysJ*	sulfite reductase (NADPH) flavoprotein subunit alpha	-6
SAOUHSC_02982	*sasF*	hypothetical protein	-3
SAOUHSC_03001	*icaR*	ica operon transcriptional regulator IcaR	-4
SAOUHSC_03034		hypothetical protein	-3
SAOUHSC_A01081		hypothetical protein	-4
SAOUHSC_A01723		hypothetical protein	-9

Upregulated

Amino acid biosynthesis

SAOUHSC_00142	*fdh*	formate dehydrogenase	4
SAOUHSC_00421	*mccA*	hypothetical protein	5
SAOUHSC_00898	*argH*	argininosuccinate lyase	8
SAOUHSC_00899	*argG*	argininosuccinate synthase	13
SAOUHSC_01319	*thrD*	aspartate kinase	3
SAOUHSC_01321	*thrC*	threonine synthase	3
SAOUHSC_01322	*thrB*	homoserine kinase	3
SAOUHSC_01483	*aroC*	chorismate synthase	2
SAOUHSC_02830	*ddh*	D-lactate dehydrogenase	2

Biosynthesis of cofactors, prosthetic groups, and carriers

SAOUHSC_00171	*ggt*	gamma-glutamyltranspeptidase	3
SAOUHSC_00386	*ssl3*	superantigen-like protein	7
SAOUHSC_00833		hypothetical protein	4
SAOUHSC_00877		hypothetical protein	2

SAOUHSC_01041	*pdhB*	pyruvate dehydrogenase complex, E1 component subunit beta	3
SAOUHSC_01256		hypothetical protein	2
SAOUHSC_01824	*thiI*	thiamine biosynthesis protein ThiI	3
SAOUHSC_02329	*thiM*	hydroxyethylthiazole kinase	3
SAOUHSC_02330	*thiD*	phosphomethylpyrimidine kinase	4
SAOUHSC_02536	*moaA*	molybdenum cofactor biosynthesis protein A	3
SAOUHSC_02537	*mobA*	molybdopterin-guanine dinucleotide biosynthesis protein MobA	4
SAOUHSC_02538	*moaD*	molybdopterin converting factor subunit 1	5
SAOUHSC_02541	*mobB*	molybdopterin-guanine dinucleotide biosynthesis protein MobB	4
SAOUHSC_02543	*moaC*	molybdenum cofactor biosynthesis protein MoaC	8
SAOUHSC_02824		hypothetical protein	6
Cell envelope			
SAOUHSC_00157	*murQ*	N-acetylmuramic acid-6-phosphate etherase	3
SAOUHSC_00295	*nanA*	N-acetylneuraminate lyase	2
SAOUHSC_00426	*metQ2*	ABC transporter substrate-binding protein	3
SAOUHSC_00691	*uppP*	undecaprenyl pyrophosphate phosphatase	12
SAOUHSC_01338		hypothetical protein	5
SAOUHSC_01424	*murG*	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	3
SAOUHSC_01467	*pbp2*	penicillin-binding protein 2	4
SAOUHSC_01739	*lytH*	hypothetical protein	2
SAOUHSC_01856	*murC*	UDP-N-acetylmuramate--L-alanine ligase	2
SAOUHSC_02107	*murT*	UDP-N-acetylmuramyl tripeptide synthetase	3
SAOUHSC_02337	*murA1*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	3
SAOUHSC_02802	*fnbB*	fibronectin binding protein B	3
Cellular processes			
SAOUHSC_00364	*ahpF*	alkyl hydroperoxide reductase subunit F	5
SAOUHSC_00365	*ahpC*	alkyl hydroperoxide reductase subunit C	6
SAOUHSC_00773		LysM domain-containing protein	4
SAOUHSC_01693	*comEA*	hypothetical protein	4
SAOUHSC_01739	*lytH*	hypothetical protein	2
SAOUHSC_01920		hypothetical protein	4
SAOUHSC_02426		hypothetical protein	3
SAOUHSC_02691		hypothetical protein	4
SAOUHSC_02692		hypothetical protein	5
SAOUHSC_02700	*mdeA*	hypothetical protein	4
Central intermediary metabolism			

SAOUHSC_00139		hypothetical protein	4
SAOUHSC_00295	*nanA*	N-acetylneuraminate lyase	2
SAOUHSC_01504	*fer*	ferredoxin	3
SAOUHSC_02564	*ureG*	urease accessory protein UreG	2
DNA metabolism			
SAOUHSC_00001	*dnaA*	chromosomal replication initiation protein	6
SAOUHSC_00429		MutT/nudix family protein	3
SAOUHSC_00442	*dnaX*	DNA polymerase III subunits gamma and tau	5
SAOUHSC_00730	*recQ1*	ATP-dependent DNA helicase RecQ	3
SAOUHSC_00776	*uvrB*	excinuclease ABC subunit B	3
SAOUHSC_00779	*uvrB2*	excinuclease ABC subunit B	3
SAOUHSC_00794	*gapR*	glycolytic operon regulator	6
SAOUHSC_01095	*rnhC*	ribonuclease HIII	5
SAOUHSC_01222	*topA*	DNA topoisomerase I	3
SAOUHSC_01224	*xerC*	site-specific recombinase	3
SAOUHSC_01262	*recA*	recombinase A	3
SAOUHSC_01591	*xerD*	integrase/recombinase XerD	6
SAOUHSC_01663	*dnaG*	DNA primase	6
SAOUHSC_01734		recombination factor protein RarA	4
SAOUHSC_01821		hypothetical protein	3
Energy metabolism			
SAOUHSC_00219		hypothetical protein	3
SAOUHSC_00239	*rbsK*	ribokinase	3
SAOUHSC_00291		PfkB family carbohydrate kinase	5
SAOUHSC_00795	*gapA*	glyceraldehyde-3-phosphate dehydrogenase	4
SAOUHSC_01040	*pdhA*	pyruvate dehydrogenase complex, E1 component subunit alpha	3
SAOUHSC_01042	*pdhC*	branched-chain alpha-keto acid dehydrogenase subunit E2	3
SAOUHSC_01103	*sdhC*	succinate dehydrogenase cytochrome b-558 subunit	3
SAOUHSC_01278	*glpD*	aerobic glycerol-3-phosphate dehydrogenase	4
SAOUHSC_01818	*ald2*	alanine dehydrogenase	3
SAOUHSC_02500	*rplE*	50S ribosomal protein L5	4
SAOUHSC_02965	*arcC*	carbamate kinase	5
Fatty acid and phospholipid metabolism			
SAOUHSC_00336		acetyl-CoA acyltransferase	4
SAOUHSC_00661		hypothetical protein	3
SAOUHSC_01197	*plsX*	glycerol-3-phosphate acyltransferase PlsX	3
SAOUHSC_01198	*fabD*	malonyl CoA-acyl carrier protein transacylase	3
SAOUHSC_01199	*fabG*	3-oxoacyl-(acyl-carrier-protein) reductase	3
SAOUHSC_01623	*accC*	acetyl-CoA carboxylase biotin carboxylase subunit	4

SAOUHSC_01624	*accB*	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	5
SAOUHSC_01808	*accA*	acetyl-CoA carboxylase carboxyltransferase subunit alpha	2
SAOUHSC_01809	*accD*	acetyl-CoA carboxylase carboxyltransferase subunit beta	2
SAOUHSC_02336	*fabZ*	(3R)-hydroxymyristoyl-ACP dehydratase	2
Mobile and extrachromosomal element functions			
SAOUHSC_02180		phage minor structural protein	3
SAOUHSC_02185		phi PVL orf 13-like protein	5
SAOUHSC_02187		HK97 family phage protein	12
SAOUHSC_02691		hypothetical protein	4
SAOUHSC_02692		hypothetical protein	5
Protein fate			
SAOUHSC_00531		hypothetical protein	3
SAOUHSC_00902	*spsA*	signal peptidase IA	2
SAOUHSC_01225	*clpQ*	ATP-dependent protease peptidase subunit	4
SAOUHSC_01226	*hslU*	ATP-dependent protease ATP-binding subunit HslU	3
SAOUHSC_01423		hypothetical protein	2
SAOUHSC_01746	*secDF*	bifunctional preprotein translocase subunit SecD/SecF	4
SAOUHSC_02254	*groEL*	chaperonin GroEL	3
SAOUHSC_02327	*yidC*	hypothetical protein	4
SAOUHSC_02755		hypothetical protein	3
SAOUHSC_02941	*nrdG*	hypothetical protein	3
Protein synthesis			
SAOUHSC_00035	*cstA*	hypothetical protein	4
SAOUHSC_00348	*rpsF*	30S ribosomal protein S6	3
SAOUHSC_00350	*rpsR*	30S ribosomal protein S18	4
SAOUHSC_00430		hypothetical protein	3
SAOUHSC_00484	*tilS*	hypothetical protein	3
SAOUHSC_00518	*rplK*	50S ribosomal protein L11	7
SAOUHSC_00519	*rplA*	50S ribosomal protein L1	7
SAOUHSC_01211	*rplS*	50S ribosomal protein L19	8
SAOUHSC_01425		hypothetical protein	2
SAOUHSC_01741	*dtd*	D-tyrosyl-tRNA(Tyr) deacylase	3
SAOUHSC_01748	*tgt*	queuine tRNA-ribosyltransferase	3
SAOUHSC_01755	*rpmA*	50S ribosomal protein L27	7
SAOUHSC_01757	*rplU*	50S ribosomal protein L21	9
SAOUHSC_01784	*rplT*	50S ribosomal protein L20	3
SAOUHSC_01785	*rpmI*	50S ribosomal protein L35	3
SAOUHSC_01786	*infC*	translation initiation factor IF-3	4
SAOUHSC_01824	*thiI*	thiamine biosynthesis protein ThiI	3
SAOUHSC_01829	*rpsD*	30S ribosomal protein S4	6

SAOUHSC_02116	*gatB*	aspartyl/glutamyl-tRNA amidotransferase subunit B	3
SAOUHSC_02117	*gatA*	aspartyl/glutamyl-tRNA amidotransferase subunit A	3
SAOUHSC_02370		hypothetical protein	2
SAOUHSC_02501	*rplX*	50S ribosomal protein L24	5
SAOUHSC_02503	*rpsQ*	30S ribosomal protein S17	3
SAOUHSC_02504	*rpmC*	50S ribosomal protein L29	4
SAOUHSC_02505	*rplP*	50S ribosomal protein L16	4
SAOUHSC_02506	*rpsC*	30S ribosomal protein S3	5
SAOUHSC_02507	*rplV*	50S ribosomal protein L22	4
SAOUHSC_02508	*rpsS*	30S ribosomal protein S19	4
SAOUHSC_02509	*rplB*	50S ribosomal protein L2	6
SAOUHSC_02510	*rplW*	50S ribosomal protein L23	4
SAOUHSC_02511	*rplD*	50S ribosomal protein L4	5
SAOUHSC_02827		hypothetical protein	4
SAOUHSC_03053	*trmE*	tRNA modification GTPase TrmE	4
SAOUHSC_03055	*rpmH*	50S ribosomal protein L34	10
Purines, pyrimidines, nucleosides, and nucleotides			
SAOUHSC_00019	*purA*	adenylosuccinate synthetase	11
SAOUHSC_01171	*pyrF*	orotidine 5'-phosphate decarboxylase	5
SAOUHSC_01172	*pyrE*	orotate phosphoribosyltransferase	4
SAOUHSC_01330	*guaC*	guanosine 5'-monophosphate oxidoreductase	6
SAOUHSC_02941	*nrdG*	hypothetical protein	3
SAOUHSC_02942	*nrdD*	anaerobic ribonucleoside triphosphate reductase	4
Regulatory functions			
SAOUHSC_00096		GntR family transcriptional regulator	2
SAOUHSC_01228	*codY*	transcriptional repressor CodY	4
SAOUHSC_01320	*hom*	homoserine dehydrogenase	5
SAOUHSC_01850	*ccpA*	catabolite control protein A	3
SAOUHSC_02799	*sarT*	accessory regulator T	8
SAOUHSC_03027		hypothetical protein	4
SAOUHSC_03046		helix-turn-helix domain-containing protein	5
Signal transduction			
SAOUHSC_00213		hypothetical protein	4
Transcription			
SAOUHSC_00524	*rpoB*	DNA-directed RNA polymerase subunit beta	3
SAOUHSC_01662	*sigA*	RNA polymerase sigma factor RpoD	3
SAOUHSC_03054	*rnpA*	ribonuclease P	13
Transport and binding proteins			
SAOUHSC_00105	*phnD*	phosphonate ABC transporter substrate-binding protein	11

SAOUHSC_00136	*ssuB*	hypothetical protein	10
SAOUHSC_00137	*ssuA*	hypothetical protein	10
SAOUHSC_00138	*ssuC*	hypothetical protein	6
SAOUHSC_00167		peptide ABC transporter ATP-binding protein	4
SAOUHSC_00168		hypothetical protein	17
SAOUHSC_00169		peptide ABC transporter permease	12
SAOUHSC_00241	*rbsU*	hypothetical protein	6
SAOUHSC_00282	*brnQ2*	branched-chain amino acid transport system II carrier protein	5
SAOUHSC_00315	*mepA*	hypothetical protein	17
SAOUHSC_00317	*glpT*	glycerol-3-phosphate transporter	6
SAOUHSC_00880		hypothetical protein	53
SAOUHSC_00928	*opp-4A*	oligopeptide ABC transporter substrate-binding protein	12
SAOUHSC_01340		hypothetical protein	5
SAOUHSC_01346	*opuD1*	glycine betaine transporter	12
SAOUHSC_01656	*znuB*	hypothetical protein	3
SAOUHSC_01657	*znuC*	ABC transporter	3
SAOUHSC_02108	*ftnA*	ferritin	12
SAOUHSC_02321		hypothetical protein	10
SAOUHSC_02622	*gltS*	sodium/glutamate symporter	3
SAOUHSC_02660	*cobI*	hypothetical protein	3
SAOUHSC_02700	*mdeA*	hypothetical protein	4
SAOUHSC_02704		hypothetical protein	2
SAOUHSC_02750		hypothetical protein	5
SAOUHSC_02864	*feoB*	ferrous iron transport protein B	11
SAOUHSC_02866		hypothetical protein	3
SAOUHSC_02874	*copZ*	cation transporter E1-E2 family ATPase	3
SAOUHSC_02937	*cudT*	choline transporter	3

Unknown TIGRFAM main role

SAOUHSC_00135		hypothetical protein	4
SAOUHSC_00141		hypothetical protein	2
SAOUHSC_00170		RGD-containing lipoprotein	7
SAOUHSC_00173	*azoR*	azoreductase	4
SAOUHSC_00240	*rbsD*	D-ribose pyranase	3
SAOUHSC_00268	*esaD*	hypothetical protein	5
SAOUHSC_00269	*esaG*	hypothetical protein	3
SAOUHSC_00270		hypothetical protein	2
SAOUHSC_00290		hypothetical protein	2
SAOUHSC_00296	*nanK*	ROK family protein	5
SAOUHSC_00300	*geh*	lipase	3
SAOUHSC_00301		hypothetical protein	4
SAOUHSC_00302		hypothetical protein	17
SAOUHSC_00309		hypothetical protein	2
SAOUHSC_00314	*mepR*	hypothetical protein	3
SAOUHSC_00316	*mepB*	hypothetical protein	3
SAOUHSC_00334		hypothetical protein	2
SAOUHSC_00367	*tcyP*	hypothetical protein	3

SAOUHSC_00390	*ssl5*	superantigen-like protein 5	5
SAOUHSC_00392	*ssl7*	superantigen-like protein 7	4
SAOUHSC_00401		hypothetical protein	3
SAOUHSC_00402	*lpl3*	hypothetical protein	11
SAOUHSC_00404	*lpl8*	hypothetical protein	8
SAOUHSC_00420		hypothetical protein	203
SAOUHSC_00422	*mccB*	trans-sulfuration enzyme family protein	3
SAOUHSC_00428		hypothetical protein	3
SAOUHSC_00434	*gltC*	LysR family transcriptional regulator	4
SAOUHSC_00436	*gltD*	glutamate synthase subunit beta	3
SAOUHSC_00465	*veg*	hypothetical protein	7
SAOUHSC_00487	*hslO*	Hsp33-like chaperonin	4
SAOUHSC_00532	*kbl*	2-amino-3-ketobutyrate coenzyme A ligase	3
SAOUHSC_00544	*sdrC*	fibrinogen-binding protein SdrC	3
SAOUHSC_00545	*sdrD*	fibrinogen-binding protein SdrD	4
SAOUHSC_00581		hypothetical protein	2
SAOUHSC_00582		hypothetical protein	4
SAOUHSC_00603		hypothetical protein	3
SAOUHSC_00604		hypothetical protein	5
SAOUHSC_00617		hypothetical protein	3
SAOUHSC_00618		hypothetical protein	4
SAOUHSC_00652	*fhuC*	iron compound ABC transporter ATP-binding protein	3
SAOUHSC_00653	*fhuB*	ferrichrome transport permease FhuB	5
SAOUHSC_00654	*fhuG*	ferrichrome ABC transporter permease	3
SAOUHSC_00694	*mgrA*	hypothetical protein	3
SAOUHSC_00729		ABC transporter ATP-binding protein	6
SAOUHSC_00734		hypothetical protein	5
SAOUHSC_00796	*pgk*	phosphoglycerate kinase	3
SAOUHSC_00846		hypothetical protein	3
SAOUHSC_00878	*ndh2*	hypothetical protein	4
SAOUHSC_00881		hypothetical protein	10
SAOUHSC_00918		truncated MHC class II analog protein	6
SAOUHSC_00991		hypothetical protein	3
SAOUHSC_01030		hypothetical protein	4
SAOUHSC_01043	*pdhD*	dihydrolipoamide dehydrogenase	2
SAOUHSC_01112	*flr*	formyl peptide receptor-like 1 inhibitory protein	4
SAOUHSC_01173		hypothetical protein	2
SAOUHSC_01193	*fakA*	hypothetical protein	2
SAOUHSC_01196	*fapR*	fatty acid biosynthesis transcriptional regulator	4
SAOUHSC_01255		hypothetical protein	3
SAOUHSC_01283		hypothetical protein	3
SAOUHSC_01344		hypothetical protein	3
SAOUHSC_01392		ABC transporter ATP-binding protein	2
SAOUHSC_01447	*ebh*	hypothetical protein	3
SAOUHSC_01462	*gpsB*	hypothetical protein	5
SAOUHSC_01480		hypothetical protein	2

SAOUHSC_01499		hypothetical protein	2
SAOUHSC_01520		SLT orf 488-like protein	5
SAOUHSC_01521		SLT orf 636-like protein	6
SAOUHSC_01590		hypothetical protein	3
SAOUHSC_01592	*fur*	transcriptional regulator Fur	4
SAOUHSC_01603		hypothetical protein	3
SAOUHSC_01604		hypothetical protein	5
SAOUHSC_01723		hypothetical protein	3
SAOUHSC_01724		hypothetical protein	4
SAOUHSC_01732	*cymR*	hypothetical protein	3
SAOUHSC_01733		hypothetical protein	6
SAOUHSC_01756		hypothetical protein	8
SAOUHSC_01762		hypothetical protein	4
SAOUHSC_01843	*isdH*	hypothetical protein	17
SAOUHSC_01873	*sasC*	hypothetical protein	5
SAOUHSC_02073		hypothetical protein	4
SAOUHSC_02080		bacteriophage L54a antirepressor	3
SAOUHSC_02167	*scn*	hypothetical protein	2
SAOUHSC_02171	*sak*	staphylokinase	3
SAOUHSC_02184		phi PVL orf 14-like protein	22
SAOUHSC_02255	*groES*	co-chaperonin GroES	3
SAOUHSC_02272		hypothetical protein	3
SAOUHSC_02322		hypothetical protein	12
SAOUHSC_02331	*tenA*	hypothetical protein	5
SAOUHSC_02333	*sceD*	transglycosylase SceD	3
SAOUHSC_02404	*fmtB*	hypothetical protein	3
SAOUHSC_02406		hypothetical protein	3
SAOUHSC_02407	*cdaA*	hypothetical protein	3
SAOUHSC_02436		hypothetical protein	3
SAOUHSC_02460		hypothetical protein	2
SAOUHSC_02462		hypothetical protein	9
SAOUHSC_02463	*hysA*	hyaluronate lyase	5
SAOUHSC_02498	*rpsH*	30S ribosomal protein S8	3
SAOUHSC_02512	*rplC*	50S ribosomal protein L3	5
SAOUHSC_02540	*moaE*	molybdopterin converting factor moa	5
SAOUHSC_02571	*ssaA*	secretory antigen	10
SAOUHSC_02587	*spdB*	hypothetical protein	4
SAOUHSC_02628		hypothetical protein	2
SAOUHSC_02659		hypothetical protein	3
SAOUHSC_02703	*gpmA*	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	6
SAOUHSC_02737		epimerase/dehydratase	9
SAOUHSC_02798	*sasG*	hypothetical protein	3
SAOUHSC_02825		hypothetical protein	10
SAOUHSC_02826		hypothetical protein	5
SAOUHSC_02828		hypothetical protein	6
SAOUHSC_02873	*copA*	cation transporter E1-E2 family ATPase	4
SAOUHSC_02883		LysM domain-containing protein	3
SAOUHSC_02892		hypothetical protein	5
SAOUHSC_02893		hypothetical protein	5

SAOUHSC_02894		hypothetical protein	5
SAOUHSC_02963	*clfB*	clumping factor B	5
SAOUHSC_03022		hypothetical protein	6
SAOUHSC_03049	*noc*	hypothetical protein	4
SAOUHSC_A00332		hypothetical protein	5
SAOUHSC_A01912		hypothetical protein	5
SAOUHSC_T00026	*trnaH*	tRNA-His	3
SAOUHSC_T00030	*trnaL*	tRNA-Leu	3