



# Dual regulation of the small RNA MicC and the quiescent porin OmpN in response to antibiotic stress in *Escherichia coli*

**Supplementary Data 1.** Screening of *micC* expression by  $\beta$ -galactosidase assay using preloaded 96-well Phenotype MicroArrays™ plates (Biolog PM11 to PM19) for bacterial chemical susceptibility. Two technical replicates were assayed, and the Miller units for each compound were calculated at 30 minutes. The fold activity was calculated based on the Miller units obtained from cells grown standard conditions, in the absence of inducer.

|                     | PM11C  | PM12B  |                                       |
|---------------------|--------|--------|---------------------------------------|
| Amikacin            | Orange | Yellow | Penicillin G                          |
| Chlortetracycline   | Yellow | Yellow | Tetracycline                          |
| Lincomycin          | Yellow | Yellow | Carbenicillin                         |
| Amoxicillin         | Yellow | Orange | Oxacillin                             |
| Cloxacillin         | Yellow | Yellow | Penimepicycline                       |
| Lomefloxacin        | Yellow | Yellow | Polymyxin B                           |
| Bleomycin           | Yellow | Yellow | Paromomycin                           |
| Colistin            | Red    | Yellow | Vancomycin                            |
| Minocycline         | Red    | Yellow | D,L-Serine hydroxamate                |
| Capreomycin         | Yellow | Yellow | Sisomicin                             |
| Demeclocycline      | Yellow | Yellow | Sulfamethazine                        |
| Nafcillin           | Yellow | Yellow | Novobiocin                            |
| Cefazolin           | Yellow | Yellow | 2,4-Diamino-6,7- diisopropylpteridine |
| Enoxacin            | Yellow | Yellow | Sulfadiazine                          |
| Nalidixic acid      | Yellow | Red    | Benzethonium chloride                 |
| Chloramphenicol     | Yellow | Yellow | Tobramycin                            |
| Erythromycin        | Orange | Yellow | Sulfathiazole                         |
| Neomycin            | Yellow | Yellow | 5-Fluoroorotic acid                   |
| Ceftriaxone         | Yellow | Yellow | Spectinomycin                         |
| Gentamicin          | Yellow | Yellow | Sulfamethoxazole                      |
| Potassium tellurite | Yellow | Yellow | L-Aspartic- $\beta$ - hydroxamate     |
| Cephalothin         | Yellow | Yellow | Spiramycin                            |
| Kanamycin           | Yellow | Yellow | Rifampicin                            |
| Ofloxacin           | Yellow | Red    | Dodecyltrimethyl ammonium bromide     |

  

|                                     | PM13B  | PM14A  |                              |
|-------------------------------------|--------|--------|------------------------------|
| Ampicillin                          | Yellow | Orange | Acriflavine                  |
| Dequalinium chloride                | Orange | Yellow | Furaltadone                  |
| Nickel chloride                     | Yellow | Yellow | Sanguinarine                 |
| Azlocillin                          | Yellow | Yellow | 9-Aminoacridine              |
| 2, 2'-Dipyridyl                     | Yellow | Yellow | Fusaric acid                 |
| Oxolinic acid                       | Yellow | Orange | Sodium arsenate              |
| 6-Mercaptopurine                    | Yellow | Yellow | Boric Acid                   |
| Doxycycline                         | Orange | Yellow | 1-Hydroxypyridine -2- thione |
| Potassium chromate                  | Yellow | Yellow | Sodium cyanate               |
| Cefuroxime                          | Orange | Yellow | Cadmium chloride             |
| 5-Fluorouracil                      | Yellow | Red    | Iodoacetate                  |
| Rolitetracycline                    | Orange | Yellow | Sodium dichromate            |
| Cytosine-1-beta D-arabinofuranoside | Yellow | Yellow | Cefoxitin                    |
| Geneticin (G418)                    | Yellow | Yellow | Nitrofurantoin               |
| Ruthenium red                       | Yellow | Yellow | Sodium metaborate            |
| Cesium chloride                     | Yellow | Yellow | Chloramphenicol              |
| Glycine                             | Yellow | Yellow | Piperacillin                 |
| Thallium (I) acetate                | Yellow | Orange | Sodium metavanadate          |
| Cobalt chloride                     | Yellow | Yellow | Chelerythrine                |
| Manganese chloride                  | Yellow | Yellow | Carbenicillin                |
| Trifluoperazine                     | Orange | Yellow | Sodium nitrite               |
| Cupric chloride                     | Yellow | Yellow | EGTA                         |
| Moxalactam                          | Yellow | Red    | Promethazine                 |
| Tylosin                             | Yellow | Orange | Sodium orthovanadate         |

|                                      | PM15B | PM16A |                                     |
|--------------------------------------|-------|-------|-------------------------------------|
| Procaine                             |       |       | Cefotaxime                          |
| Guanidine hydrochloride              |       |       | Phosphomycin                        |
| Cefmetazole                          |       |       | 5-Chloro-7-iodo- 8-hydroxyquinoline |
| D-Cycloserine                        |       |       | Norfloxacin                         |
| EDTA                                 |       |       | Sulfanilamide                       |
| 5,7-Dichloro- 8- hydroxyquinaldine   |       |       | Trimethoprim                        |
| 5,7-Dichloro-8- hydroxyquinoline     |       |       | Dichlofluanid                       |
| Fusidic acid                         |       |       | Protamine sulfate                   |
| 1,10- Phenanthroline                 |       |       | Cetylpyridinium chloride            |
| Pheomycin                            |       |       | 1-Chloro -2,4- dinitrobenzene       |
| Domiphen bromide                     |       |       | Diamide                             |
| Nordihydroguaia retic acid           |       |       | Cinoxacin                           |
| Alexidine                            |       |       | Streptomycin                        |
| 5-Nitro-2- furaldehyde semicarbazone |       |       | 5-Azacytidine                       |
| Methyl viologen                      |       |       | Rifamycin SV                        |
| 3, 4-Dimethoxybenzyl alcohol         |       |       | Potassium tellurite                 |
| Oleandomycin                         |       |       | Sodium selenite                     |
| Puromycin                            |       |       | Aluminum sulfate                    |
| CCCP                                 |       |       | Chromium chloride                   |
| Sodium azide                         |       |       | Ferric chloride                     |
| Menadione                            |       |       | L-Glutamic-glydroxamate             |
| 2-Nitroimidazole                     |       |       | Glycine hydroxamate                 |
| Hydroxyurea                          |       |       | Chloroxyleneol                      |
| Zinc chloride                        |       |       | Sorbic acid                         |

|  | PM17A | PM18C |   |
|--|-------|-------|---|
| D-Serine                               |       |       | Ketoprofen                              |
| $\beta$ -ChloroL-alanine hydrochloride |       |       | Sodium pyrophosphate decahydrate        |
| Thiosalicylic acid                     |       |       | Thiamphenicol                           |
| Sodium salicylate                      |       |       | Trifluorothymidin                       |
| Hygromycin B                           |       |       | Pipemidic Acid                          |
| Ethionamide                            |       |       | Azathioprine                            |
| 4-Aminopyridine                        |       |       | Poly-L-lysine                           |
| Sulfachloropyridazine                  |       |       | Sulfisoxazole                           |
| Sulfamonomethoxine                     |       |       | Pentachlorophenol                       |
| Oxycarboxin                            |       |       | Sodium m-arsenite                       |
| 3-Amino-1,2,4- triazole                |       |       | Sodium bromate                          |
| Chlorpromazine                         |       |       | Lidocaine                               |
| Niaproof                               |       |       | Sodium metasilicate                     |
| Compound 48/80                         |       |       | Sodium m-periodate                      |
| Sodium tungstate                       |       |       | Antimony (III) chloride                 |
| Lithium chloride                       |       |       | Semicarbazide                           |
| DL-Methionine hydroxamate              |       |       | Tinidazole                              |
| Tannic acid                            |       |       | Aztreonam                               |
| Chlorambucil                           |       |       | Triclosan                               |
| Cefamandole nafate                     |       |       | 3,5-Diamino- 1,2,4-triazole (Guanazole) |
| Cefoperazone                           |       |       | Myricetin                               |
| Cefsulodin                             |       |       | 5-fluoro-5'- deoxyuridine               |
| Caffeine                               |       |       | 2-Phenylphenol                          |
| Phenylarsine oxide                     |       |       | Plumbagin                               |

|  | PM19B  | PM20B  |                    |
|--|--------|--------|--------------------|
| Josamycin                              | Yellow | Orange | Amitriptyline      |
| Gallic acid                            | Yellow | Yellow | Apramycin          |
| Coumarin                               | Yellow | Yellow | Benserazide        |
| Methyltrioctylammonium chloride        | Red    | Red    | Orphenadrine       |
| Harmane                                | Yellow | Yellow | D,L-Propranolol    |
| 2,4-Dinitrophenol                      | Yellow | Yellow | Tetrazolium violet |
| Chlorhexidine                          | Yellow | Red    | Thioridazine       |
| Umbelliferone                          | Yellow | Orange | Atropine           |
| Cinnamic acid                          | Yellow | Yellow | Ornidazole         |
| Disulphiram                            | Yellow | Yellow | Proflavine         |
| Iodonitro Tetrazolium Violet           | Yellow | Yellow | Ciprofloxacin      |
| Phenyl- methylsulfonyl fluoride (PMSF) | Yellow | Yellow | 18-Crown-6 ether   |
| FCCP                                   | Orange | Orange | Crystal violet     |
| D,L-Thioctic Acid                      | Yellow | Yellow | Dodine             |
| Lawsone                                | Yellow | Orange | Hexachlorophene    |
| Phenethicillin                         | Orange | Yellow | 4-Hydroxycoumarin  |
| Blasticidin S                          | Yellow | Yellow | Oxytetracycline    |
| Sodium caprylate                       | Orange | Red    | Pridinol           |
| Lauryl sulfobetaine                    | Red    | Yellow | Captan             |
| Dihydrostreptomycin                    | Yellow | Yellow | 3,5-Dinitrobenzene |
| Hydroxylamine                          | Yellow | Yellow | 8-Hydroxyquinoline |
| Hexamine cobalt (III) chloride         | Yellow | Yellow | Patulin            |
| Thioglycerol                           | Yellow | Yellow | Tolyfluanid        |
| Polymyxin B                            | Orange | Yellow | Troleandomycin     |

|               |           |
|---------------|-----------|
| Red           | > 10 fold |
| Orange        | 6-10 fold |
| Yellow-Orange | 3-6 fold  |
| Yellow        | 1-3 fold  |

**Supplementary Data 2.** Fifteen compounds were selected to investigate their effects on MicC and OmpN. 5 (nordihydroguaiaretic acid, thioridazine HCl, benzethonium chloride, promethazine HCl, colistin, and chlorpromazine HCl) of them were identified as inducers of the micC- and ompN-lacZ fusions by using Phenotype Microarrays plates (Biolog PM11 to PM19). First, minimal inhibitory concentrations (MICs,  $\mu\text{g/ml}$ ) were determined as described in the Materials and Methods section. Range of concentrations for each compound was then chosen according to the MIC for the measurements of  $\beta$ -galactosidase activities by using the microtiter plate method. The final working concentrations were chosen as the lowest concentrations that produced the maximal changes to the  $\beta$ -galactosidase activities.

| Compound                  | MIC ( $\mu\text{g/ml}$ ) | Range of concentration tested ( $\mu\text{g/ml}$ ) | Chosen concentration ( $\mu\text{g/ml}$ ) |
|---------------------------|--------------------------|--|---|
| Benzalkonium chloride     | 0.2                      | 0.1-0.8  | 0.4                                       |
| Nordihydroguaiaretic acid | 100                      | 50-400   | 200                                       |
| Doripenem                 | 0.2                      | 0.1-0.8  | 0.4                                       |
| Thioridazine HCl          | 100                      | 50-400   | 200                                       |
| Benzethonium chloride     | 100                      | 50-400   | 200                                       |
| Meropenem                 | 1                        | 0.5-8  | 2   |
| Imipenem                  | 1.25                     | 0.3125-10  | 1.25                                      |
| Promethazine HCl          | 200                      | 200-1600   | 800                                       |
| Biapenem                  | 0.32                     | 0.8-3.2  | 3.2                                       |
| Polymyxin B               | 1                        | 1-16   | 2   |
| Colistin                  | 1                        | 1-16   | 2   |
| Ceftazidime               | 1                        | 1-16   | 4   |
| Cefepime                  | 1                        | 1-16   | 2   |
| Ertapenem                 | 1                        | 1-16   | 6   |
| Chlorpromazine HCl        | 200                      | 200-1600   | 400                                       |

**Supplementary Data 3.** The expression of OmpN was evaluated in laboratory and clinical strains of *E. coli* by Western blot analysis. Briefly, cells were grown overnight in LB broth, spun down, resuspended in 1× Laemmli buffer. Samples were heated for 5 min at 95 °C prior to protein separation by SDS-PAGE and electrotransferred onto nitrocellulose blotting membranes. The strains were selected based on their OmpF and OmpC expression profile, as well as their resistance towards β-lactam (CAZ, ceftazidime and ERT, ertapenem) and other (CM, chloramphenicol). These data are from Pagès *et al.* [2].

| <i>E. coli</i> strains | Porin expression |      |      | MIC (µg/ml) |      |       | β-lactamases    |
|------------------------|------------------|------|------|-------------|------|-------|-----------------|
|                        | OmpF             | OmpC | OmpN | CAZ         | ERT  | CM    | AmpC            |
| AG100                  | +                | +    | -    | 0.5         | 0.02 | 0.125 | AmpC            |
| AG100A                 | +                | +    | -    | 0.5         | 0.02 | 0.031 | AmpC            |
| AG100Atet              | +                | +    | -    | 1           | 0.02 | 0.5   | AmpC            |
| ARS100                 | -                | -    | -    | 1           | 2    | 32    | AmpC            |
| ARS108                 | -                | -    | -    | 32          | 4    | >128  | CTX-M-15        |
| ARS144                 | +                | +    | -    | 1024        | 0.5  | >128  | CTX-M-15, DHA-1 |
| ARS150                 | +                | +    | -    | 512         | 2    | >128  | CTX-M-15, TEM-1 |
| ARS183                 | +                | +    | -    | 64          | 1    | >128  | CTX-M-14, TEM-1 |
| ARS237                 | +                | +    | -    | 128         | 4    | >128  | CTX-M-14, TEM-1 |
| ARS273                 | -                | +    | -    | 128         | 8    | >128  | CTX-M-15        |

+: presence; -: absence

**Supplementary Data 4.** Partial *pfo(ybdK)-micC-ompN* genetic region. *micC* (in green) is transcribed clockwise on the *E. coli* chromosome on the opposite strand of the adjacent *ompN* and *pfo* (former *ybdK*) genes (in blue). Relevant characteristics of the region are indicated, such as the *pfo* stop codon (blue star) and the *ompN* start codon (blue, +1). Primer extension analysis of MicC RNA [1] allowed the precise position of the MicC transcriptional start (green, +1), -10 and -35 promoter sequences (green, underlined). The position of the *ompN* -10 and -35 promoter sequences was predicted by using the BPROM software, available at <http://www.softberry.com/berry.phtml?topic=bprom>. Positions and sequences of the primers used for PCR-amplifying fragments of this region are indicated by arrows.

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AATTCGCAGCAGCCAGAAGTGGCAGAACAGTTATGGAAAGATGCTGCAGCTGATTTGCAAAAACGCTATGACTTC
                                     SD5
                                     Pfo (YbdK) → *
CTGGCACAAATGGCCGGAAGCGGAAAAAGCAACACCGATTAAATGCTCTGGATAAGGATTATCCAATTCTAA

AAAAAAGCCCGACGACTGTTCTGGGCTGTCTTTTATATGTTGGAAAATCAGTGGCAATGCAATGGCCCAACA
                                     MicC ← +1 SD2 SD4
GAAAATAAAATCTGTGACAATAAAGGCATATAACCCGCGCAGAATAACGTATAGCAAATAAAAGTATAATTTTT
                                     -10 -35
ATTTTGTATGTATTATCTTCGATACATTCACCAGACTTATTATCATTTCAGAGGAATTATTTGATTAAGGTTTTTA
                                     -35 -10
CTTAAGGCGTAACAAATGATATTTATCGGCTAACTGAACTTCTCCTTTAGGATGTTTTCACTCCCGAAATGGGG
                                     SD1
                                     +1 → OmpN
ATAAAAGGCAAATAAAATAACTAAAGGATTTATTCAATGAAAAGCAAAGTACTGGCACTTTTAATTCCTGCCCTG
SD3

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## References

- Chen, S.; Zhang, A.; Blyn, L.B.; Storz, G. MicC, a second small-RNA regulator of Omp protein expression in *Escherichia coli*. *J. Bacteriol.* **2004**, *186*, 6689–6697.
- Pagès, J.M.; Peslier, S.; Keating, T.A.; Lavigne, J.P.; Nichols, W.W. Role of the outer membrane and porins in susceptibility of β-lactamase-producing *Enterobacteriaceae* to ceftazidime-avibactam. *Antimicrob. Agents Chemother.* **2015**, *60*, 1349–1359.

