

Supplementary Information

Peptide-nucleotide Antibiotic Microcin C Is a Potent Inducer of Stringent Response and Persistence in Both Sensitive and Producing Cells

Julia Piskunova^{1,2,3}, *Etienne Maisonneuve*³, *Elsa Germain*³, *Kenn Gerdes*³, and
Konstantin Severinov^{1,2,4*}

¹Skolkovo Institute of Science and Technology, Skolkovo 143025, Russia;

²Institute of Gene Biology, Russian Academy of Sciences, Moscow 119334, Russia;

³Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark

⁴Waksman Institute for Microbiology, Rutgers, The State University of New Jersey, Piscataway, NJ 08854

*To whom correspondence should be addressed. Tel: +1 848 445 6096; Fax: +1 848 445 5735; Email: severik@waksman.rutgers.edu

SUPPLEMENTARY MATERIALS AND METHODS

Plate assay for McC production

100 μ l of fresh night culture of *E. coli* BL21 (DE3) cells was mixed with 5 ml 0.7% (soft) LB agar and poured over a 1.5% LB agar plate. After drying, 5 μ l aliquots of tested cell cultures were applied on the plate surface. Plates were incubated at 30 °C for 10 h to allow the lawn to grow, and the sizes of growth inhibition zones formed around antibiotic solution drops were measured.

FIGURE LEGENDS

Figure S1 (related to Figure 5). McC producing culture has an increased frequency of (p)ppGpp “ON” cells. Statistical distribution of the fluorescence level of individual cells from

(A) MG1655 *rpoS::mCherry*,

(B) MG1655 pp70 *rpoS::mCherry*, collected in exponential culture and analyzed by fluorescence microscopy.

Panels show the fluorescence distribution as a histogram consisting of 80 repartition bins (Log scale). The different colors represent three individual experiments including a total of more than 120,000 cells. For each strain, “n” represents the number of cells analyzed in individual sets. Right panel represents the same distribution using a logarithmic Y axis. Arrow points at the threshold applied to discriminate ON cells from OFF cells (n represents the number of ON cells observed in each set).

Figure S2. Antibacterial activity of McC produced by wild type or isogenic $\Delta relA$ or $\Delta relA spoT$ strains.

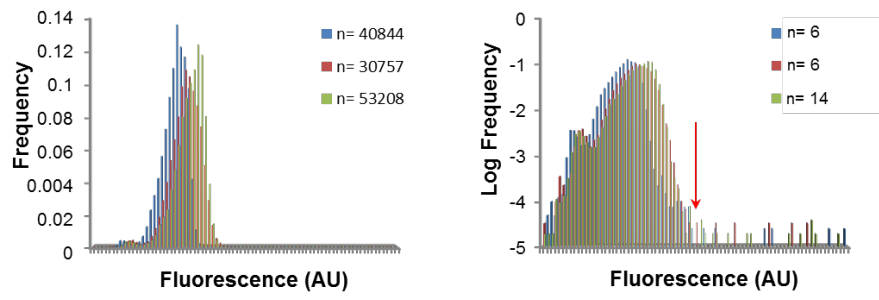
Aliquots of cultured medium after 2 (A) or 4 (B) hours of growth of McC producing strains were placed over a growing lawn of McC-sensitive *E. coli* cells. Growth inhibition zones are seen as clear circles on the turbid surface of cell lawn. See SUPPLEMENTARY MATERIALS AND METHODS for details.

Figure S3. Antibacterial activity of McC produced by wild type or $\Delta yejA$ strains.

Aliquots of cultured medium after 2-hour growth of McC producing strains were placed over a growing lawn of McC-sensitive *E. coli* cells. Growth inhibition zones are seen as clear circles on the turbid surface of cell lawn. See SUPPLEMENTARY MATERIALS AND METHODS for details.

Figure S1

A



B

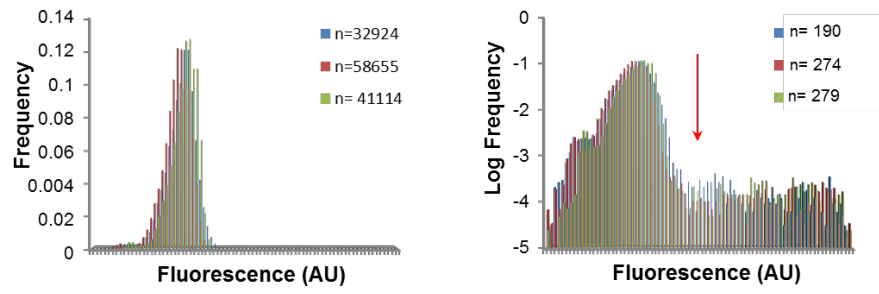
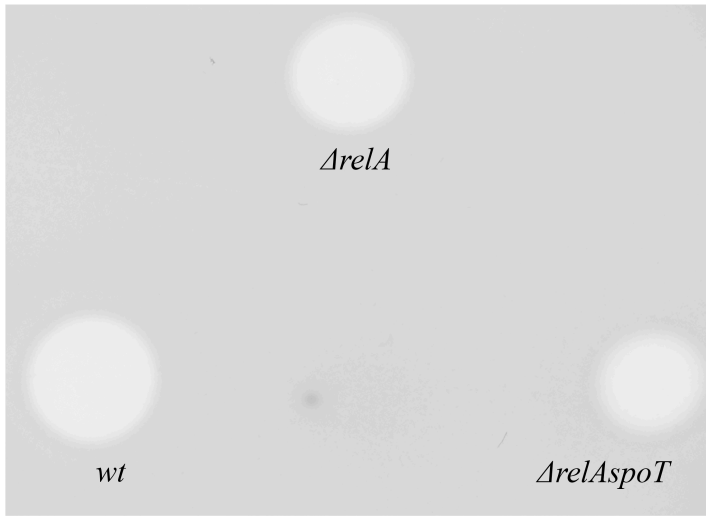


Figure S2

A



B

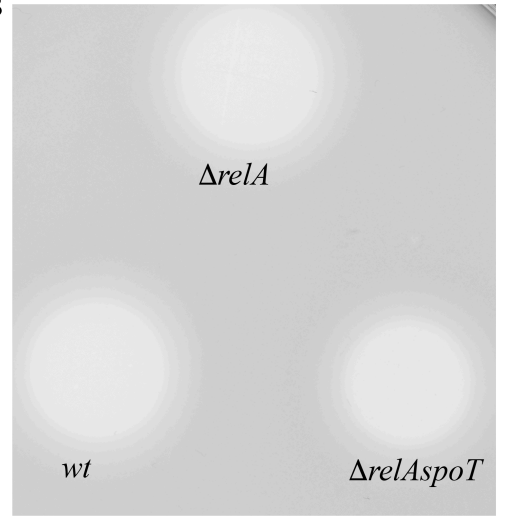


Figure S3

