

Supplementary Material:

**Identification and characterization of new
resistance-conferring SGI1s (*Salmonella* genomic island 1) in
*Proteus mirabilis***

Luyao Bie¹, Meng Fang¹, Zhiqiang Li², Mingyu Wang^{1*} and Hai Xu^{1*}

¹ State Key Laboratory of Microbial Technology, Microbial Technology Research Institute, Shandong University, Qingdao, China

² Advanced Research Center for Optics, Shandong University, Qingdao, China

* Correspondence:

Mingyu Wang wangmingyu@sdu.edu.cn, Hai Xu haixu@sdu.edu.cn.

1 SUPPLEMENTARY DATA

2 SUPPLEMENTARY TABLES AND FIGURES

2.1 Figures

Figure S1. Model of the site-specific integration and excision of SGI1. Black arrows represent chromosomal genes of *P. mirabilis*. SGI1 is represented in grey rectangle. SGI1 integrates specifically at the 3' end of the chromosomal *trmE* gene (*attB*). The direct repeats (DR-L/DR-R) are formed by the recombination between the chromosomal *attB* site and the SGI1 *attP* site. Primers (circ1/circ2) used for detection of the circular SGI1 form are indicated by horizontal arrows.

Figure S2. Detection of SGI1 and circular extrachromosomal form of SGI1, **(A)** Lane1-6, left junction by PmLJ1/LJR1(JN16, JN29, JN47, JN40, JN48, N); Lane7-12, S026 (JN16, JN29, JN47, JN40, JN48, N); Lane13-18, right junction by 104-RJ/PmRJ1 (JN16, JN29, JN47, JN40, JN48, N), **(B)** Lane1-3, right junction by 104-RJ/hipA-R1 (JN40, JN48, N); Lane 4-6, right junction by 104-RJ/MP-R1 (JN40, JN48, N); Lane7-12, S044 (JN16, JN29, JN47, JN40, JN48, N); Lane13-18, circular extrachromosomal form of SGI1 by circ1/circ2 (JN16, JN29, JN47, JN40, JN48, N). N, negative control; M, marker.

Figure S3. Resistance of *P. mirabilis* JN16 and JN48 to chromate.

Figure S4. Detection of pR55 and SGI1 in transconjugants. **(A)** Lane 1-4, *parA* of pR55 in *P. mirabilis* (JN29, JN40, JN47, JN48); Lane 5-8, *xis* of SGI1s in transconjugants (SGI1-*PmCAU*, SGI1-*PmABB*, SGI1-*PmJN40*, SGI1-*PmJN48*); Lane 9-12, S044 of SGI1s in transconjugants (SGI1-*PmCAU*, SGI1-*PmABB*, SGI1-*PmJN40*, SGI1-*PmJN48*). **(B)** Lane 1-3, *orf4*, *orf16*, and *orf17* of SGI1-*PmJN40*; Lane 4-6, PMI3015, PMI3046, and *chrA* of SGI1-*PmJN48*. P, positive control; N, negative control; M, marker (2000bp, 1500bp, 1000bp, 750bp, 500bp, 250bp, 100bp).

2.2 Tables

Table S1. Primers used for PCR in this study.

| Primer | Sequence (5'-3') | Target gene | Reference |
|---------------------------------------|-----------------------|-------------|--------------|
| Detection and location of SGI1 | | | |
| PmLJ1 | ACACCTACAACAAGGCTATC | <i>thdF</i> | (Boyd, 2007) |
| LJ-R1 | AGTTCTAAAGGTTCGTAGTCG | <i>int</i> | (Boyd, 2001) |
| 104-RJ | TGACGAGCTGAAGCGAATTG | S044 | (Boyd, 2001) |
| PmRJ1 | GATGCACACTGAGTTGATAG | <i>hipB</i> | (Boyd, 2007) |

| | | | |
|---------|---------------------|-------------|------------|
| hipA-R1 | GCCGCTAATAGGTCAAAGG | <i>hipA</i> | (Bi, 2011) |
| MP-R1 | CCCAAATGGCAGAAAGAAT | PMI3124 | (Bi, 2011) |
| S026-F | TGGCTACTGCAGAACAC | S026 | this study |
| S026-R | TACCTGACTGCCTCTAG | | |
| S044-F | AGCATTCCGATGATGTG | S044 | this study |
| S044-R | TTCTCCCTGATCTTCATC | | |

Mapping of the SGI1 backbones

| | | | |
|-----------|--------------------|------------------|--------------------------------|
| intSGI1-F | GGTATCAGTAAACAAGCG | <i>int</i> -S005 | (Siebor and Neuwirth, 2013) |
| S005-outF | CACTCTCTCGAATCATCC | | |
| rep-outR | CGACTTGGCACATGATTG | <i>rep</i> -S005 | (Siebor and Neuwirth, 2013) |
| S005-F | CATGACGAGGTTTGTG | | |
| S005-R | GTAAACGATGCAGGATC | S005-S010 | (Siebor and Neuwirth, 2013) |
| S010-F | CGAGCTGAATATAGACGC | | |
| S010-R | CGGATACCGTTGAGGCGA | S010-S011 | (Siebor and Neuwirth, 2013) |
| S011-F | TTATTCCGTCGGTGATGC | | |
| S011-outR | ATACACGAGCCAACAAGC | S011-S014 | (Siebor and Neuwirth, 2013) |
| S014-R | AGATGATCGGACATAGCG | | |
| S014-F | TGAGTCTGTGGCATGAAG | S014-S020 | (Siebor and Neuwirth, 2013) |
| S020-mF | TCTGCGGTTCGAGGAATC | | |
| S020-R | GTGAGCGTAGTGGTGC | S020-S024 | (Siebor and Neuwirth, 2013) |
| S024-outF | GGCTCATACCAATACAGC | | |
| S024-R | ATGATCCTGGCATGTTCG | S024-S025 | (Siebor and Neuwirth, 2013) |
| S025-mF | TCTTGGGATCCTGATTGG | | |
| S025-R | CACTCTATTCCCACCG | S025-S026 | (Siebor and Neuwirth, 2013) |
| S026-F | TGGCTACTGCAGAACAC | | |

Mapping of the MDR regions

| | | | |
|------------|--------------------|---------------------|--------------------------------|
| S026-outR | CAACCTGAAGGGCAACAG | S026- <i>aadA2</i> | (Siebor and Neuwirth, 2013) |
| aadA2-R | CTCGCCTTCACAAAGCG | | |
| aadA2-outF | ATAAAACGCCAACCTGCC | <i>aadA2</i> -S044 | (Siebor and Neuwirth, 2013) |
| S044-outR | TCGCTCGATAACTCTCTC | | |
| S026-outR | CAACCTGAAGGGCAACAG | S026- <i>dfrA1</i> | (Siebor and Neuwirth, 2013) |
| dfrA1-R | ACCCTTTGCCAGATTG | | |
| orfC-outF | TGGTTCCCTGCTTGATTG | <i>orfC</i> -S044 | (Siebor and Neuwirth, 2013) |
| S044-outR | TCGCTCGATAACTCTCTC | | |
| S026-outR | CAACCTGAAGGGCAACAG | S026- <i>aacCA5</i> | (Siebor and Neuwirth, 2013) |
| aacCA5-R | TTTACTCTGCTCAACCGC | | |
| aadA7-outF | CGCCAAAGGATGTTGCTG | <i>aadA7</i> -S044 | (Siebor and Neuwirth, 2013) |
| S044-outR | TCGCTCGATAACTCTCTC | | |
| S026-outR | CAACCTGAAGGGCAACAG | S026-PSE1 | (Siebor and Neuwirth, 2013) |
| PSE1-R | CTTATCAGCGCGACTGTG | | |

| | | | |
|--|----------------------|----------------------|-----------------|
| PSE1-outF | TACAGCAGTTGTGGAG | | (Siebor and |
| S044-outR | TCGCTCGATAACTCTCTC | <i>blaPSE-1-S044</i> | Neuwirth, 2013) |
| IS1359F | TGGGTCCACCACATTGATAC | IS1359 | |
| IS1359R | AAGGGAGATGGCTAACGAA | | this study |
| Detection of the circular extrachromosomal form of SGI1 | | | |
| circ1 | AGCAAAATCGTGAGAAGGGA | <i>attP</i> | (Doublet, 2005) |
| circ2 | TGATGAGACACCTGACGAGC | | |
| Detection of the plasmid R55 | | | |
| parAF | AAAAGTAATCAGCTTCGCCA | <i>parA</i> | |
| parAR | TAGCCCACCTCTTAATAG | | this study |
| Detection of the transconjugants of SGI1s | | | |
| xisF | TCAGGCGGTAGATGTATG | <i>xis</i> | |
| xisR | GAAAGTGAATGGCGGAAT | | this study |
| orf4F | TTTACATAGCGGCGAATC | <i>orf4</i> | |
| orf4R | TTAGCCAGTTGAGCACC | | this study |
| orf16F | AGTCCAAGATGCCGTAG | <i>orf16</i> | |
| orf16R | ATGTGGTAGCGATAAGC | | this study |
| orf17F | TCCAAAGAGTAAAGCCAGTA | <i>orf17</i> | |
| orf17R | AGCAGGTAAGGTGCGATG | | this study |
| 3015F | ACACCACGCTCTGTTCCA | PMI3015 | |
| 3015R | TGTTTATTGCCCTACGC | | this study |
| 3046F | CTCTAACGACCACCTAT | PMI3046 | |
| 3046R | AAAGTCTCAGATGGCAAG | | this study |
| chrAF | TAACCCTACCGATGACAGCA | <i>chrA</i> | |
| chrAR | GATAGCAATCACGCCAAC | | this study |

References

- Bi, S., Yan, H., Chen, M., Zhang, Z., Shi, L., and Wang, H. (2011). New variant *Salmonella* genomic island 1-U in *Proteus mirabilis* clinical and food isolates from South China. *J. Antimicrob. Chemoth.* 66, 1178-1179. doi: 10.1093/jac/dkr030
- Boyd, D. A., Peters, G. A., Cloeckaert, A., Boumedine, K. S., Chaslus-Dancla, E., Imberechts, H., et al. (2001). Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. *J. Bacteriol.* 183, 5725-5732. doi: 10.1128/JB.183.19.5725-5732.2001
- Boyd, D. A., Shi, X., Hu, Q. H., Ng, L. K., Doublet, B., Cloeckaert, A., et al. (2007). *Salmonella* Genomic Island 1 (SGI1), variant SGI1-I, and new variant SGI1-O in *Proteus mirabilis* clinical and food isolates from China. *Antimicrob. Agents.*

- Chemoth.* 52, 340-344. doi: 10.1128/AAC.00902-07
- Doublet, B., Boyd, D. A., Mulvey, M. R., and Cloeckaert, A. (2005). The *Salmonella* genomic island 1 is an integrative mobilizable element. *Mol. Microbiol.* 55, 1911-1924. doi: 10.1111/j.1365-2958.2005.04520.x
- Siebor, E., and Neuwirth, C. (2013). Emergence of *Salmonella* genomic island 1 (SGI1) among *Proteus mirabilis* clinical isolates in Dijon, France. *J. Antimicrob. Chemoth.* 68, 1750-1756. doi: 10.1093/jac/dkt100