

The ABC-Type Efflux Pump MacAB Is Involved in Protection of *Serratia marcescens* against Aminoglycoside Antibiotics, Polymyxins, and Oxidative Stress

Shirshikova T.V., Sierra-Bakhshi C.G., Kamaletdinova L.K., Matrosova L.E., Khabipova N.N., Evtugyn V.G., Khilyas I.V., Danilova I.V., Mardanova A.M., Sharipova M.R., Bogomolnaya L.M.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

Serratia marcescens is an emerging pathogen with increasing clinical importance due to its intrinsic resistance to several classes of antibiotics. The chromosomally encoded drug efflux pumps contribute to antibiotic resistance and represent a major challenge for the treatment of bacterial infections. The ABC-type efflux pump MacAB was previously linked to macrolide resistance in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. The role of the MacAB homolog in antibiotic resistance of *S. marcescens* is currently unknown. We found that an *S. marcescens* mutant lacking the MacAB pump did not show increased sensitivity to the macrolide antibiotic erythromycin but was significantly more sensitive to aminoglycoside antibiotics and polymyxins. We also showed that, in addition to its role in drug efflux, the MacAB efflux pump is required for swimming motility and biofilm formation. We propose that the motility defect of the DmacAB mutant is due, at least in part, to the loss of functional flagella on the bacterial surface. Furthermore, we found that the promoter of the MacAB efflux pump was active during the initial hours of growth in laboratory medium and that its activity was further elevated in the presence of hydrogen peroxide. Finally, we demonstrate a complete loss of DmacAB mutant viability in the presence of peroxide, which is fully restored by complementation. Thus, the *S. marcescens* MacAB efflux pump is essential for survival during oxidative stress and is involved in protection from polymyxins and aminoglycoside antibiotics.

<http://dx.doi.org/10.1128/mSphere.00033-21>

Keywords

antibiotic resistance, efflux pump, MacAB, multidrug efflux pumps, oxidative stress, physiological role, *Serratia*, *Serratia marcescens*

References

- [1] 1. Mahlen SD. 2011. *Serratia* infections: from military experiments to current practice. *Clin Microbiol Rev* 24:755-791. <https://doi.org/10.1128/CMR.00017-11>.
- [2] 2. WHO. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. [https:// www.who.int/medicines/publications/global-priority-list-antibiotic-resistant - bacteria/en/](https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/).

- [3] 3. Blair JMA, Bavro VN, Ricci V, Modi N, Cacciotto P, Kleinekathfer U, Ruggerone P, Vargiu AV, Baylay AJ, Smith HE, Brandon Y, Galloway D, Piddock LJV. 2015. AcrB drug-binding pocket substitution confers clinically relevant resistance and altered substrate specificity. *Proc Natl Acad Sci U S A* 112:3511-3516. <https://doi.org/10.1073/pnas.1419939112>.
- [4] 4. Poole K. 2007. Efflux pumps as antimicrobial resistance mechanisms. *Ann Med* 39:162-176. <https://doi.org/10.1080/07853890701195262>.
- [5] 5. Duque E, Segura A, Mosqueda G, Ramos JL. 2001. Global and cognate regulators control the expression of the organic solvent efflux pumps TtgABC and TtgDEF of *Pseudomonas putida*. *Mol Microbiol* 39:1100-1106. <https://doi.org/10.1046/j.1365-2958.2001.02310.x>.
- [6] 6. Ganas P, Mihasan M, Igloi GL, Brandsch R. 2007. A two-component small multidrug resistance pump functions as a metabolic valve during nicotine catabolism by *Arthrobacter nicotinovorans*. *Microbiology (Reading)* 153:1546-1555. <https://doi.org/10.1099/mic.0.2006/004234-0>.
- [7] 7. Horiyama T, Nishino K. 2014. AcrB, AcrD, and MdtABC multidrug efflux systems are involved in enterobactin export in *Escherichia coli*. *PLoS One* 9:e108642. <https://doi.org/10.1371/journal.pone.0108642>.
- [8] 8. Dietrich LE, Price-Whelan A, Petersen A, Whiteley M, Newman DK. 2006. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Mol Microbiol* 61:1308-1321. <https://doi.org/10.1111/j.1365-2958.2006.05306.x>.
- [9] 9. Aendekerk S, Diggle SP, Song Z, Hoiby N, Cornelis P, Williams P, Camara M. 2005. The MexGHI-OpmD multidrug efflux pump controls growth, antibiotic susceptibility and virulence in *Pseudomonas aeruginosa* via 4-quinolone-dependent cell-to-cell communication. *Microbiology (Reading)* 151:1113-1125. <https://doi.org/10.1099/mic.0.27631-0>.
- [10] 10. Nishino K, Latifi T, Groisman EA. 2006. Virulence and drug resistance roles of multidrug efflux systems of *Salmonella enterica* serovar Typhimurium. *Mol Microbiol* 59:126-141. <https://doi.org/10.1111/j.1365-2958.2005.04940.x>.
- [11] 11. Ruiz C, Levy SB. 2014. Regulation of *acrAB* expression by cellular metabolites in *Escherichia coli*. *J Antimicrob Chemother* 69:390-399. <https://doi.org/10.1093/jac/dkt352>.
- [12] 12. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, Luisi BF. 2018. Multidrug efflux pumps: structure, function and regulation. *Nat Rev Microbiol* 16:523-539. <https://doi.org/https://doi.org/10.1038/s41579-018-0048-6>.
- [13] 13. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, Luisi BF. 2018. Author correction: multidrug efflux pumps: structure, function and regulation. *Nat Rev Microbiol* 16:577-577. <https://doi.org/10.1038/s41579-018-0060-x>.
- [14] 14. Kumar A, Worobec EA. 2005. Cloning, sequencing, and characterization of the SdeAB multidrug efflux pump of *Serratia marcescens*. *Antimicrob Agents Chemother* 49:1495-1501. <https://doi.org/10.1128/AAC.49.4.1495-1501.2005>.
- [15] 15. Chen J, Kuroda T, Huda MN, Mizushima T, Tsuchiya T. 2003. An RND-type multidrug efflux pump SdeXY from *Serratia marcescens*. *J Antimicrob Chemother* 52:176-179. <https://doi.org/10.1093/jac/dkg308>.
- [16] 16. Maseda H, Hashida Y, Konaka R, Shirai A, Kourai H. 2009. Mutational upregulation of a resistance-nodulation-cell division-type multidrug efflux pump, SdeAB, upon exposure to a biocide, cetylpyridinium chloride, and antibiotic resistance in *Serratia marcescens*. *Antimicrob Agents Chemother* 53:5230-5235. <https://doi.org/10.1128/AAC.00631-09>.
- [17] 17. Dalvi SD, Worobec EA. 2012. Gene expression analysis of the SdeAB multidrug efflux pump in antibiotic-resistant clinical isolates of *Serratia marcescens*. *Indian J Med Microbiol* 30:302-307. <https://doi.org/10.4103/0255-0857.99491>.
- [18] 18. Minato Y, Shahcheraghi F, Ogawa W, Kuroda T, Tsuchiya T. 2008. Functional gene cloning and characterization of the SsmE multidrug efflux pump from *Serratia marcescens*. *Biol Pharm Bull* 31:516-519. <https://doi.org/10.1248/bpb.31.516>.
- [19] 19. Shahcheraghi F, Minato Y, Chen J, Mizushima T, Ogawa W, Kuroda T, Tsuchiya T. 2007. Molecular cloning and characterization of a multidrug efflux pump, SmfY, from *Serratia marcescens*. *Biol Pharm Bull* 30:798-800. <https://doi.org/10.1248/bpb.30.798>.
- [20] 20. Matsuo T, Chen J, Minato Y, Ogawa W, Mizushima T, Kuroda T, Tsuchiya T. 2008. SmdAB, a heterodimeric ABC-Type multidrug efflux pump, in *Serratia marcescens*. *J Bacteriol* 190:648-654. <https://doi.org/10.1128/JB.01513-07>.
- [21] 21. Mardanova AM, Bogomol'naia LM, Romanova ID, Sharipova MR. 2014. Efflux systems in *Serratia marcescens*. *Mikrobiologiya* 83:3-14. (In Russian.)
- [22] 22. Kobayashi N, Nishino K, Yamaguchi A. 2001. Novel macrolide-specific ABC-type efflux transporter in *Escherichia coli*. *J Bacteriol* 183:5639-5644. <https://doi.org/10.1128/JB.183.19.5639-5644.2001>.
- [23] 23. Yamanaka H, Kobayashi H, Takahashi E, Okamoto K. 2008. MacAB is involved in the secretion of *Escherichia coli* heat-stable enterotoxin II. *J Bacteriol* 190:7693-7698. <https://doi.org/10.1128/JB.00853-08>.

- [24] 24. Rouquette-Loughlin CE, Balthazar JT, Shafer WM. 2005. Characterization of the MacA-MacB efflux system in *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 56:856-860. <https://doi.org/10.1093/jac/dki333>.
- [25] 25. Lin YT, Huang YW, Liou RS, Chang YC, Yang TC. 2014. MacABCsm, an ABCtype tripartite efflux pump of *Stenotrophomonas maltophilia* involved in drug resistance, oxidative and envelope stress tolerances and biofilm formation. *J Antimicrob Chemother* 69:3221-3226. <https://doi.org/10.1093/jac/dku317>.
- [26] 26. Zheng JX, Lin ZW, Sun X, Lin WH, Chen Z, Wu Y, Qi GB, Deng QW, Qu D, Yu ZJ. 2018. Overexpression of OqxAB and MacAB efflux pumps contributes to eravacycline resistance and heteroresistance in clinical isolates of *Klebsiella pneumoniae*. *Emerg Microbes Infect* 7:139. <https://doi.org/10.1038/s41426-018-0141-y>.
- [27] 27. Bogomolnaya LM, Tilwawala R, Eifenbein JR, Cirillo JD, Andrews- Polymenis HL. 2020. Linearized siderophore products secreted via MacAB efflux pump protect *Salmonella enterica* serovar Typhimurium from oxidative stress. *mBio* 11:e00528-20. <https://doi.org/10.1128/mBio.00528-20>.
- [28] 28. Bogomolnaya LM, Andrews KD, Talamantes M, Maple A, Ragoza Y, Vazquez-Torres A, Andrews-Polymenis H. 2013. The ABC-type efflux pump MacAB protects *Salmonella enterica* serovar typhimurium from oxidative stress. *mBio* 4:e00630-13. <https://doi.org/10.1128/mBio.00630-13>.
- [29] 29. Khilyas IV, Tursunov KA, Shirshikova TV, Kamaletdinova LK, Matrosova LE, Desai PT, McClelland M, Bogomolnaya LM. 2019. Genome sequence of pigmented siderophore-producing strain *Serratia marcescens* SM6. *Microbiol Resour Announc* 8e00247-19. <https://doi.org/10.1128/MRA.00247-19>.
- [30] 30. Nishino K, Nikaido E, Yamaguchi A. 2009. Regulation and physiological function of multidrug efflux pumps in *Escherichia coli* and *Salmonella*. *Biochim Biophys Acta* 1794:834-843. <https://doi.org/10.1016/j.bbapap.2009.02.002>.
- [31] 31. CLSI. 2020. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI, Wayne, PA.
- [32] 32. Moradigaravand D, Boinett CJ, Martin V, Peacock SJ, Parkhill J. 2016. Recent independent emergence of multiple multidrug-resistant *Serratia marcescens* clones within the United Kingdom and Ireland. *Genome Res* 26:1101-1109. <https://doi.org/10.1101/gr.205245.116>.
- [33] 33. Fuste E, Galisteo GJ, Jover L, Vinuesa T, Villa TG, Vinas M. 2012. Comparison of antibiotic susceptibility of old and current *Serratia*. *Future Microbiol* 7:781-786. <https://doi.org/10.2217/fmb.12.40>.
- [34] 34. Olaitan AO, Morand S, Rolain JM. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* 5:643. <https://doi.org/10.3389/fmicb.2014.00643>.
- [35] 35. Lin QY, Tsai YL, Liu MC, Lin WC, Hsueh PR, Liaw SJ. 2014. *Serratia marcescens* arn, a PhoP-regulated locus necessary for polymyxin B resistance. *Antimicrob Agents Chemother* 58:5181-5190. <https://doi.org/10.1128/AAC.00013-14>.
- [36] 36. Honeycutt JD, Wenner N, Li Y, Brewer SM, Massis LM, Brubaker SW, Chairatana P, Owen SV, Canals R, Hinton JCD, Monack DM. 2020. Genetic variation in the MacAB-TolC efflux pump influences pathogenesis of invasive *Salmonella* isolates from Africa. *PLoS Pathog* 16:e1008763. <https://doi.org/10.1371/journal.ppat.1008763>.
- [37] 37. Baugh S, Ekanayaka AS, Piddock LJ, Webber MA. 2012. Loss of or inhibition of all multidrug resistance efflux pumps of *Salmonella enterica* serovar Typhimurium results in impaired ability to form a biofilm. *J Antimicrob Chemother* 67:2409-2417. <https://doi.org/10.1093/jac/dks228>.
- [38] 38. Mitrofanova O, Mardanova A, Evtugyn V, Bogomolnaya L, Sharipova M. 2017. Effects of *Bacillus* serine proteases on the bacterial biofilms. *Biomed Res Int* 2017:8525912. <https://doi.org/10.1155/2017/8525912>.
- [39] 39. Nizamutdinova E, Shirshikova TV, Mardanova AM, Sharipova MR, Bogomol'naya LM. 2016. Effect of mutations in extracellular nuclease on the characteristics of the pigmented and nonpigmented *Serratia marcescens* strains. *Mikrobiologiya* 85:36-41. (In Russian.)
- [40] 40. Nesme J, Cecillon S, Delmont TO, Monier JM, Vogel TM, Simonet P. 2014. Large-scale metagenomic-based study of antibiotic resistance in the environment. *Curr Biol* 24:1096-1100. <https://doi.org/10.1016/j.cub.2014.03.036>.
- [41] 41. Noronha MF, Lacerda Junior GV, Gilbert JA, de Oliveira VM. 2017. Taxonomic and functional patterns across soil microbial communities of global biomes. *Sci Total Environ* 609:1064-1074. <https://doi.org/10.1016/j.scitotenv.2017.07.159>.
- [42] 42. Miryala SK, Ramaiah S. 2019. Exploring the multi-drug resistance in *Escherichia coli* O157:H7 by gene interaction network: a systems biology approach. *Genomics* 111:958-965. <https://doi.org/10.1016/j.ygeno.2018.06.002>.
- [43] 43. Vallet-Gely I, Novikov A, Augusto L, Liehl P, Bolbach G, Pechy-Tarr M, Cosson P, Keel C, Caroff M, Lemaitre B. 2010. Association of hemolytic activity of *Pseudomonas entomophila*, a versatile soil bacterium, with cyclic lipopeptide production. *Appl Environ Microbiol* 76:910-921. <https://doi.org/10.1128/AEM.02112-09>.
- [44] 44. Cho H, Kang H. 2012. The PseEF efflux system is a virulence factor of *Pseudomonas syringae* pv. *syringae*. *J Microbiol* 50:79-90. <https://doi.org/10.1007/s12275-012-1353-9>.
- [45] 45. Sandner-Miranda L, Vinuesa P, Cravioto A, Morales-Espinosa R. 2018. The genomic basis of intrinsic and acquired antibiotic resistance in the genus *Serratia*. *Front Microbiol* 9:828. <https://doi.org/10.3389/fmicb.2018.00828>.

- [46] 46. Lin MF, Lin YY, Tu CC, Lan CY. 2017. Distribution of different efflux pump genes in clinical isolates of multidrug-resistant *Acinetobacter baumannii* and their correlation with antimicrobial resistance. *J Microbiol Immunol Infect* 50:224-231. <https://doi.org/10.1016/j.jmii.2015.04.004>.
- [47] 47. Sulavik MC, Houseweart C, Cramer C, Jiwani N, Murgolo N, Greene J, DiDomenico B, Shaw KJ, Miller GH, Hare R, Shimer G. 2001. Antibiotic susceptibility profiles of *Escherichia coli* strains lacking multidrug efflux pump genes. *Antimicrob Agents Chemother* 45:1126-1136. <https://doi.org/10.1128/AAC.45.4.1126-1136.2001>.
- [48] 48. Padilla E, Llobet E, Domenech-Sanchez A, Martinez-Martinez L, Bengoechea JA, Alberti S. 2010. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. *Antimicrob Agents Chemother* 54:177-183. <https://doi.org/10.1128/AAC.00715-09>.
- [49] 49. Fehlner-Gardiner CC, Valvano MA. 2002. Cloning and characterization of the *Burkholderia vietnamiensis* norM gene encoding a multi-drug efflux protein. *FEMS Microbiol Lett* 215:279-283. <https://doi.org/10.1111/j.1574-6968.2002.tb11403.x>.
- [50] 50. Loutet SA, Valvano MA. 2011. Extreme antimicrobial peptide and polymyxin B resistance in the genus *Burkholderia*. *Front Microbiol* 2:159.
- [51] 51. Srinivasan VB, Singh BB, Priyadarshi N, Chauhan NK, Rajamohan G. 2014. Role of novel multidrug efflux pump involved in drug resistance in *Klebsiella pneumoniae*. *PLoS One* 9:e96288. <https://doi.org/10.1371/journal.pone.0096288>.
- [52] 52. Huang YW, Liou RS, Lin YT, Huang HH, Yang TC. 2014. A linkage between SmelJK efflux pump, cell envelope integrity, and sigmaE-mediated envelope stress response in *Stenotrophomonas maltophilia*. *PLoS One* 9:e111784. <https://doi.org/10.1371/journal.pone.0111784>.
- [53] 53. Lin MF, Lin YY, Lan CY. 2017. Contribution of EmrAB efflux pumps to colistin resistance in *Acinetobacter baumannii*. *J Microbiol* 55:130-136. <https://doi.org/10.1007/s12275-017-6408-5>.
- [54] 54. Krishnamoorthy G, Weeks JW, Zhang Z, Chandler CE, Xue H, Schweizer HP, Ernst RK, Zgurskaya HI. 2019. Efflux pumps of *Burkholderia thailandensis* control the permeability barrier of the outer membrane. *Antimicrob Agents Chemother* 63:e00956-19. <https://doi.org/10.1128/AAC.00956-19>.
- [55] 55. Aendekerk S, Ghysels B, Cornelis P, Baysse C. 2002. Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. *Microbiology (Reading)* 148:2371-2381. <https://doi.org/10.1099/00221287-148-8-2371>.
- [56] 56. Buckner MM, Blair JM, La Ragione RM, Newcombe J, Dwyer DJ, Ivens A, Piddock LJ. 2016. Beyond antimicrobial resistance: evidence for a distinct role of the AcrD efflux pump in *Salmonella* biology. *mBio* 7:e01916-16. <https://doi.org/10.1128/mBio.01916-16>.
- [57] 57. Lin YT, Huang YW, Chen SJ, Chang CW, Yang TC. 2015. The SmeYZ efflux pump of *Stenotrophomonas maltophilia* contributes to drug resistance, virulence-related characteristics, and virulence in mice. *Antimicrob Agents Chemother* 59:4067-4073. <https://doi.org/10.1128/AAC.00372-15>.
- [58] 58. Perez-Varela M, Corral J, Aranda J, Barbe J. 2019. Roles of efflux pumps from different superfamilies in the surface-associated motility and virulence of *Acinetobacter baumannii* ATCC 17978. *Antimicrob Agents Chemother* 63:e02190-18. <https://doi.org/10.1128/AAC.02190-18>.
- [59] 59. Lewis K. 2001. Riddle of biofilm resistance. *Antimicrob Agents Chemother* 45:999-1007. <https://doi.org/10.1128/AAC.45.4.999-1007.2001>.
- [60] 60. Ito A, Taniuchi A, May T, Kawata K, Okabe S. 2009. Increased antibiotic resistance of *Escherichia coli* in mature biofilms. *Appl Environ Microbiol* 75:4093-4100. <https://doi.org/10.1128/AEM.02949-08>.
- [61] 61. Kvist M, Hancock V, Klemm P. 2008. Inactivation of efflux pumps abolishes bacterial biofilm formation. *Appl Environ Microbiol* 74:7376-7382. <https://doi.org/10.1128/AEM.01310-08>.
- [62] 62. Matsumura K, Furukawa S, Ogihara H, Morinaga Y. 2011. Roles of multidrug efflux pumps on the biofilm formation of *Escherichia coli* K-12. *Biocontrol Sci* 16:69-72. <https://doi.org/10.4265/bio.16.69>.
- [63] 63. Grkovic S, Brown MH, Skurray RA. 2001. Transcriptional regulation of multidrug efflux pumps in bacteria. *Semin Cell Dev Biol* 12:225-237. <https://doi.org/10.1006/scdb.2000.0248>.
- [64] 64. Sun J, Deng Z, Yan A. 2014. Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. *Biochem Biophys Res Commun* 453:254-267. <https://doi.org/10.1016/j.bbrc.2014.05.090>.
- [65] 65. Munch R, Hiller K, Grote A, Scheer M, Klein J, Schobert M, Jahn D. 2005. Virtual Footprint and PRODORIC: an integrative framework for regulon prediction in prokaryotes. *Bioinformatics* 21:4187-4189. <https://doi.org/10.1093/bioinformatics/bti635>.
- [66] 66. Pardo-Este C, Castro-Severyn J, Kruger GI, Cabezas CE, Briones AC, Aguirre C, Morales N, Baquedano MS, Sulbaran YN, Hidalgo AA, Meneses C, Poblete-Castro I, Castro-Nallar E, Valvano MA, Saavedra CP. 2019. The transcription factor ArcA modulates *Salmonella*'s metabolism in response to neutrophil hypochlorous acid-mediated stress. *Front Microbiol* 10:2754. <https://doi.org/10.3389/fmicb.2019.02754>.
- [67] 67. Imlay JA. 2015. Transcription factors that defend bacteria against reactive oxygen species. *Annu Rev Microbiol* 69:93-108. <https://doi.org/10.1146/annurev-micro-091014-104322>.

- [68] 68. Rosenberg EY, Bertenthal D, Nilles ML, Bertrand KP, Nikaido H. 2003. Bile salts and fatty acids induce the expression of *Escherichia coli* AcrAB multidrug efflux pump through their interaction with Rob regulatory protein. *Mol Microbiol* 48:1609-1619. <https://doi.org/10.1046/j.1365-2958.2003.03531.x>.
- [69] 69. Urdaneta V, Casades J. 2018. Adaptation of *Salmonella enterica* to bile: essential role of AcrAB-mediated efflux. *Environ Microbiol* 20:1405-1418. <https://doi.org/10.1111/1462-2920.14047>.
- [70] 70. Ma D, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. 1995. Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. *Mol Microbiol* 16:45-55. <https://doi.org/10.1111/j.1365-2958.1995.tb02390.x>.
- [71] 71. Turlin E, Heuck G, Simoes Brandao MI, Szili N, Mellin JR, Lange N, Wandersman C. 2014. Protoporphyrin (PPIX) efflux by the MacAB-TolC pump in *Escherichia coli*. *Microbiologyopen* 3:849-859. <https://doi.org/10.1002/mbo3.203>.
- [72] 72. Khilyas IV, Shirshikova TV, Matrosova LE, Sorokina AV, Sharipova MR, Bogomolnaya LM. 2016. Production of siderophores by *Serratia marcescens* and the role of MacAB efflux pump in siderophores secretion. *BioNanoSci* 6:480-482. <https://doi.org/10.1007/s12668-016-0264-3>.
- [73] 73. Friend JC, Hilligoss DM, Marquesen M, Ulrick J, Estwick T, Turner ML, Cowen EW, Anderson V, Holland SM, Malech HL. 2009. Skin ulcers and disseminated abscesses are characteristic of *Serratia marcescens* infection in older patients with chronic granulomatous disease. *J Allergy Clin Immunol* 124:164-166. <https://doi.org/10.1016/j.jaci.2009.04.009>.
- [74] 74. Kamaletdinova LK, Nizamutdinova EK, Shirshikova TV, Skipina IM, Bogomolnaya LM. 2016. Inactivation of chromosomal genes in *Serratia marcescens*. *BioNanoScience* 6:376-378. <https://doi.org/10.1007/s12668-01-0249-2>.
- [75] 75. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* 97:6640-6645. <https://doi.org/10.1073/pnas.120163297>.
- [76] 76. Takagi T, Kisumi M. 1985. Isolation of a versatile *Serratia marcescens* mutant as a host and molecular cloning of the aspartase gene. *J Bacteriol* 161:1-6. <https://doi.org/10.1128/JB.161.1.1-6.1985>.
- [77] 77. Evans TJ, Crow MA, Williamson NR, Orme W, Thomson NR, Komitopoulou E, Salmond GP. 2010. Characterization of a broad-host-range flagellum-dependent phage that mediates high-efficiency generalized transduction in, and between, *Serratia* and *Pantoea*. *Microbiology (Reading)* 156:240-247. <https://doi.org/10.1099/mic.0.032797-0>.
- [78] 78. Shirshikova TV, Morozova OV, Kamaletdinova LK, Sharipova MR, Bogomolnaya LM. 2016. Generalized bacteriophage transduction in *Serratia marcescens*. *BioNanoScience* 6:487-489. <https://doi.org/10.1007/s12668-016-0268-z>.
- [79] 79. Uzzau S, Figueroa-Bossi N, Rubino S, Bossi L. 2001. Epitope tagging of chromosomal genes in *Salmonella*. *Proc Natl Acad Sci U S A* 98:15264-15269. <https://doi.org/10.1073/pnas.261348198>.
- [80] 80. Wang RF, Kushner SR. 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* 100:195-199. [https://doi.org/10.1016/0378-1119\(91\)90366-J](https://doi.org/10.1016/0378-1119(91)90366-J).
- [81] 81. Bogomolnaya LM, Aldrich L, Ragoza Y, Talamantes M, Andrews KD, McClelland M, Andrews-Polymeris HL. 2014. Identification of novel factors involved in modulating motility of *Salmonella enterica* serotype Typhimurium. *PLoS One* 9:e111513. <https://doi.org/10.1371/journal.pone.0111513>.
- [82] 82. Ellermeier CD, Janakiraman A, Slauch JM. 2002. Construction of targeted single copy lac fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. *Gene* 290:153-161. [https://doi.org/10.1016/s0378-1119\(02\)00551-6](https://doi.org/10.1016/s0378-1119(02)00551-6).
- [83] 83. Zhang X, Bremer H. 1995. Control of the *Escherichia coli* *rrnB* P1 promoter strength by ppGpp. *J Biol Chem* 270:11181-11189. <https://doi.org/10.1074/jbc.270.19.11181>.