Send Orders for Reprints to reprints@benthamscience.net

The Open Microbiology Journal, **2014**, *8*, **51-58** **51**

Open Access

Conservation of the Low-shear Modeled Microgravity Response in Enterobacteriaceae and Analysis of the *trp* Genes in this Response

Anjali Soni[1,2], Laura O'Sullivan[1,3], Laura N. Quick[1, 4], C. Mark Ott[5], Cheryl A. Nickerson[6] and James W. Wilson[*,1]

[1]*Villanova University, Biology Department, 800 Lancaster Avenue, Villanova, PA 19085*

[2]*Virginia Commonwealth University, School of Dentistry, Richmond, VA23298*

[3]*University of Pennsylvania,School of Veterinary Medicine, Philadelphia, PA 19104*

[4]*Children's Hospital of Philadelphia, Philadelphia, PA 19104*

[5]*NASA/Johnson Space Center, Habitability and Environmental Factors Division, Houston, TX77058*

[6]*Arizona State University, Biodesign Institute, Center for Infectious Diseases and Vaccinology, Tempe, AZ85281*

Abstract: Low fluid shear force, including that encountered in microgravity models, induces bacterial responses, but the range of bacteria capable of responding to this signal remains poorly characterized. We systematically analyzed a range of Gram negative Enterobacteriaceae for conservation of the low-shear modeled microgravity (LSMMG) response using phenotypic assays, qPCR, and targeted mutations. Our results indicate LSMMG response conservation across Enterobacteriacae with potential variance in up- or down-regulation of a given response depending on genus. Based on the data, we analyzed the role of the *trp* operon genes and the TrpR regulator in the LSMMG response using targeted mutations in these genes in *S.* Typhimurium and *E. coli*. We found no alteration of the LSMMG response compared to WT in these mutant strains under the conditions tested here. To our knowledge, this study is first-of-kind for *Citrobacter, Enterobacter*, and *Serratia*, presents novel data for *Escherichia*, and provides the first analysis of *trp* genes in LSMMG responses. This impacts our understanding of how LSMMG affects bacteria and our ability to modify bacteria with this condition in the future.

Keywords: Enterobacteriaceae, environmental response, low shear modeled microgravity, rotating wall vessel, *Salmonella* Typhimurium.

INTRODUCTION

A large body of studies aimed at characterizing the effects of low fluid shear force environments on bacterial cells,including the microgravity environment of spaceflight and ground-based rotating wall vessel (RWV) culture, have focused on the Gram negative enteric pathogen *Salmonella enterica* serovar Typhimurium [1-7]. Other studies have also focused on additional bacteria including *Escherichia coli* [8-15], *Pseudomonas aeruginosa* [16-18], *Yersinia pestis* [19, 20], and *Staphylococcus aureus* [15, 21, 22] with a range of different phenotypic results. However, the range of bacteria capable of responding to this condition and the potential similarities/differences in this response remain poorly characterized. Additionally, to our knowledge, a systematic, "side-by-side" study to examine the conservation of the low fluid shear response in a range of different bacterial genera using common assay conditions has not been reported in the literature. In this study,we analyzed different members of the Gram negative Enterobacteriaceae family for conservation of the low fluid shear response in the RWV using phenotypic and molecular assays to delineate the commonalities and differences of these organisms to culture in this environmental condition. Many members of the Enterobactericeae family are enteric organisms that inhabit the intestine as part of their lifecycles, and consequently, these bacteria likely encounter low fluid shear regions in their natural habitat such as the spaces between microvilli [23]. In addition, as bacterial engineering expands to include a larger range of bacteria, the use of novel growth conditions will be applied to a greater variety of genera.

The RWV culture apparatus (Fig. **1**) allows a physiologically-relevant low fluid shear force growth environment that induces a number of phenotypic responses in bacteria including altered stress resistance [1-5, 7, 12, 16, 21], increased survival in cellular and animal hosts [1, 3, 4, 7], and altered gene expression [1, 3, 5, 7, 13, 14, 17, 18]. The RWV is used to induce prokaryotic and eukaryotic cellular

*Address correspondence to this author at the Villanova University, Biology Department, 800 Lancaster Avenue, Villanova, PA 19085;
Tel: 610-519-3037; Fax: 610-519-7863;
E-mail: james.w.wilson@villanova.edu

1874-2858/14 2014 Bentham Open

Table 1. DNA oligonucleotides used in this study.

Name	Sequence
S. Typhimurium	
hfq	acaagatccgttcctgaacgcattgcgtcg
	tgttgctgtgatgggaaaccgggcgagacg
trpD	agcgcctttgtcgcggcggcctgtgga
	gttgatcagcgggccgagtacgttgaacag
E. coli	
hfq	acaagatccgttcctgaacgcactgcgtcg
	tgttactgtgatgagaaaccgggcgagacg
trpD	agtgcgtttgtcgccgcagcctgtggg
	gttaatcaatggccccagcacattgaacag
C. freundii	
hfq	acaagatccgttcctgaacgcactgcgtcg
	tgttactgtgatgagaaaccgggcgagacg
trpD	agcgcctttgttgcggcggcttgcgga
	gttaatcaacgggccgagcacgttaaacag
E. cloacae	
hfq	acaagatccgttcctgaacgcattgcgtcg
	tattgctgtgatgagataccggacgggacg
ydcI	ctgaacgaactggaacaactcacc
	catcgtattgttcatggtcgcgacctg
Normalization	
16S rRNA	gtaacggctcaccaaggcgacgatccctag
	cttcgccaccggtattcctccagatctctac
lpxC	ccgttgagcacctgaatgctgctctggcgg
	tctggcgcatgaacgcatccgcagagaagt
S. Typhimurium recombineering	
Δ*trpR*	ctcgtgtaacagtacaaacggcggtataacatgacccagccatatgaatatcctccttagttcc
	tggccgcttcgccttatccggcctacgagcaaatcaggcgtgtgtaggctggagctgcttc
Δ*trpDE*	acccacgctcgaactgttgacctgcgatgccgcctatcgggatgtgtaggctggagctgcttc
	ccaaatcgccttgtctgcgacgattttcgctaaaacggtttgcatatgaatatcctccttagttcc
E. coli recombineering	
Δ*trpR*	cccgctaacaatggcgacatattatggcccaacaatcacccatatgaatatcctccttagttcc
	gatgcgccacgtcttatcaggcctacaaaatcaatcgcttgtgtaggctggagctgcttc
Δ*trpDE*	acgtaaaagagtcgatattatcgagcagcagaatgtcagccatatgaatatcctccttagttcc
	aaccgactctcgaactgctaacctgcgaaggcgcttatcgtgtgtaggctggagctgcttc

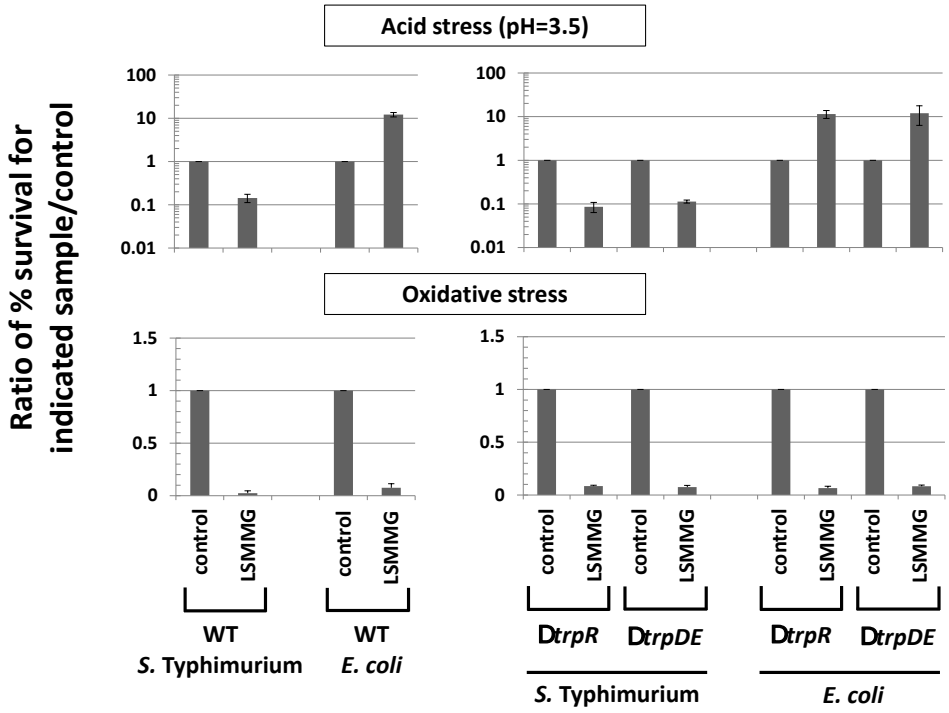

Fig. (6). Analysis of role of *trp* genes in LSMMG phenotypes. *S.* Typhimurium and *E.coli* Δ *trpR* and Δ *trpDE* strains were compared to respective WT strains for LSMMG-mediated alteration of acid and oxidative stress as described in Figs. (**3**, **4**). The data was obtained from at least three independent experiments each plated in triplicate, and the average and standard deviation are plotted. The differences between LSMMG and control were significant at p-value < 0.05.

enzyme encoded by the *trp* operon could be having a role in the LSMMG response. Therefore, we constructed a Δ*trpDE* mutation in *S.* Typhimurium and *E. coli* that knocked out expression of this enzyme, and we tested these mutants for LSMMG phenotypes compared to WT (Fig. **6**). As with the Δ*trpR* strains, we observed no effect of Δ*trpDE* on LSMMG responses in these genera indicating that the *trp* operon genes are not involved the LSMMG response as tested here.

DISCUSSION

The results presented here provide significant evidence using several strains and multiple assays that the response to the LSMMG growth environment is conserved across Enterobacteriaceae.Thus, the pathways and mechanisms used for sensing this environmental signal appear to be present in these different cells. This report also demonstrates that in a "side-by-side" study, the direction of LSMMG-induced regulation of phenotypes and gene expression can vary depending on genus. These results indicate that applications using the LSMMG environment (and related spaceflight conditions) have the potential to be targeted to a range of Entrobacteriaceae genera. Future work will focus on understanding the underlying causes of the variance in the direction of LSMMG regulation observed here and on the use of LSMMG/spaceflight to engineer bacteria in different ways.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGMENTS

We thank the Villanova University Biology Department, Sigma Xi Grants-in-Aid G20111015158058, and NASA grant NNX09AH40G for support of this work. We thank members of the Villanova Biology Department for discussions and assistance during this work.

REFERENCES

[1] Nickerson CA, Ott CM, Mister SJ, Morrow BJ, Burns-Keliher L, Pierson DL. Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar Typhimurium virulence. Infect Immun 2000; 68(6): 3147-52.

[2] Nauman EA, Ott CM, Sander E, *et al*. Novel quantitative biosystem for modeling physiological fluid shear stress on cells. Appl Environ Microbiol 2007; 73(3): 699-705.

[3] Wilson JW, Ott CM, Quick L, *et al*. Media ion composition controls regulatory and virulence response of *Salmonella* in spaceflight. PloS One 2008; 3(12): e3923.

[4] Wilson JW, Ott CM, Ramamurthy R, *et al*. Low-Shear modeled microgravity alters the *Salmonella enterica* serovar Typhimurium stress response in an RpoS-independent manner. Appl Environ Microbiol 2002; 68(11): 5408-16.

[5] Wilson JW, Ramamurthy R, Porwollik S, *et al*. Microarray analysis identifies *Salmonella* genes belonging to the low-shear modeled microgravity regulon. Proc Natl Acad Sci USA 2002; 99(21): 13807-12.

[6] Pacello F, Rotilio G, Battistoni A. Low-Shear modeled microgravity enhances *Salmonella Enterica* Resistance to hydrogen peroxide through a mechanism involving KatG and KatN. Open Microbiol J 2012; 6: 53-64.

[7] Wilson JW, Ott CM, Honer zu Bentrup K, *et al*. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proc Natl Acad Sci USA 2007; 104(41): 16299-304.

[8] Fang A, Pierson DL, Koenig DW, Mishra SK, Demain AL. Effect of simulated microgravity and shear stress on microcin B17 production by *Escherichia coli* and on its excretion into the medium. Appl Environ Microbiol 1997; 63(10): 4090-2.

[9] Fang A, Pierson DL, Mishra SK, Demain AL. Relief from glucose interference in microcin B17 biosynthesis by growth in a rotating-wall bioreactor. Lett Appl Microbiol 2000; 31(1): 39-41.

[10] Gao Q, Fang A, Pierson DL, Mishra SK, Demain AL. Shear stress enhances microcin B17 production in a rotating wall bioreactor, but ethanol stress does not. Appl Microbiol Biotechnol 2001; 56(3-4): 384-7.

[11] Lynch SV, Brodie EL, Matin A. Role and regulation of sigma S in general resistance conferred by low-shear simulated microgravity in *Escherichia coli*. J Bacteriol 2004; 186(24): 8207-12.

[12] Lynch SV, Mukundakrishnan K, Benoit MR, Ayyaswamy PS, Matin A. *Escherichia coli* biofilms formed under low-shear modeled microgravity in a ground-based system. Appl Environ Microbiol 2006; 72(12): 7701-10.

[13] Tucker DL, Ott CM, Huff S, *et al*. Characterization of *Escherichia coli* MG1655 grown in a low-shear modeled microgravity environment. BMC Microbiol 2007; 7: 15.

[14] Arunasri K, Adil M, Venu Charan K, Suvro C, Himabindu Reddy S, Shivaji S. Effect of simulated microgravity on *E. coli* K12 MG1655 growth and gene expression. PloS One 2013; 8(3): e57860.

[15] Vukanti R, Model MA, Leff LG. Effect of modeled reduced gravity conditions on bacterial morphology and physiology. BMC Microbiol 2012; 12: 4.

[16] Crabbe A, Pycke B, Van Houdt R, *et al*. Response of *Pseudomonas aeruginosa* PAO1 to low shear modelled microgravity involves AlgU regulation. Environ Microbiol 2010; 12(6): 1545-64.

[17] Crabbe A, Schurr MJ, Monsieurs P, *et al.* Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. Appl Environ Microbiol 2011; 77(4): 1221-30.

[18] Crabbe A, De Boever P, Van Houdt R, Moors H, Mergeay M, Cornelis P. Use of the rotating wall vessel technology to study the effect of shear stress on growth behaviour of *Pseudomonas aeruginosa* PA01. Environ Microbiol 2008; 10(8): 2098-110.

[19] Lawal A, Jejelowo OA, Rosenzweig JA. The effects of low-shear mechanical stress on *Yersinia pestis* virulence. Astrobiology 2010; 10(9): 881-8.

[20] Lawal A, Kirtley ML, van Lier CJ, *et al.* The effects of modeled microgravity on growth kinetics, antibiotic susceptibility, cold growth, and the virulence potential of a *Yersinia pestisymo*- a deficient mutant and its isogenic parental strain. Astrobiology 2013;13(9): 821-32.

[21] Castro SL, Nelman-Gonzalez M, Nickerson CA, Ott CM. Induction of attachment-independent biofilm formation and repression of Hfq expression by low-fluid-shear culture of *Staphylococcus aureus*. Appl Environ Microbiol 2011; 77(18): 6368-78.

[22] Rosado H, Doyle M, Hinds J, Taylor P. Low-shear modelled microgravity alters expression of virulence determinants of *Staphylococcus aureus*. Acta Astronautica 2010; 66(3-4): 408-13.

[23] Guo P, Weinstein AM, Weinbaum S. A hydrodynamic mechanosensory hypothesis for brush border microvilli. Am J Physiol Renal Physiol 2000; 279(4): F698-712.

[24] Barrila J, Radtke AL, Crabbe A, *et al*. Organotypic 3D cell culture models: using the rotating wall vessel to study host-pathogen interactions. Nature Rev Microbiol 2010; 8(11): 791-801.

[25] Nickerson CA, Ott CM, Wilson JW, *et al*. Low-shear modeled microgravity: a global environmental regulatory signal affecting bacterial gene expression, physiology, and pathogenesis. J Microbiol Methods 2003; 54(1): 1-11.

[26] Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL. Microbial responses to microgravity and other low-shear environments. Microbiol Mol Biol Rev 2004; 68(2): 345-61.

[27] Rosenzweig JA, Chopra AK. The effect of low shear force on the virulence potential of *Yersinia pestis*: new aspects that space-like growth conditions and the final frontier can teach us about a formidable pathogen. Front Cell Infect Microbiol 2012; 2: 107.

[28] Allen CA, Niesel DW, Torres AG. The effects of low-shear stress on adherent-invasive *Escherichia coli*. Environ Microbiol 2008; 10(6): 1512-25. Epub 2008/03/04.

[29] Gulig PA, Curtiss R, 3rd. Plasmid-associated virulence of *Salmonella* Typhimurium. Infect Immun 1987; 55(12): 2891-901.

[30] Blattner FR, Plunkett G, 3rd, Bloch CA, *et al*. The complete genome sequence of *Escherichia coli* K-12. Science 1997; 277(5331): 1453-62.

[31] Ayres EK, Thomson VJ, Merino G, Balderes D, Figurski DH. Precise deletions in large bacterial genomes by vector-mediated excision (VEX). The *trfA* gene of promiscuous plasmid RK2 is essential for replication in several gram-negative hosts. J Mol Biol 1993; 230(1): 174-85.

[32] O'Sullivan LE, Nickerson CA, Wilson JW. A series of IncQ-based reporter plasmids for use in a range of Gram negative genera. J Microbiol Biotechnol 2010; 20(5): 871-4.

[33] Jennings ME, Quick LN, Soni A, *et al*. Characterization of the *Salmonella enterica* serovar Typhimurium *ydcI* gene, which encodes a conserved DNA binding protein required for full acid stress resistance. J Bacteriol 2011; 193(9): 2208-17.

[34] Hommais F, Zghidi-Abouzid O, Oger-Desfeux C, *et al*. *lpxC* and *yafS* are the most suitable internal controls to normalize real time RT-qPCR expression in the phytopathogenic bacteria *Dickeya dadantii*. PloS One 2011; 6(5): e20269.

[35] Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci USA 2000; 97(12): 6640-5.

[36] Cho BK, Federowicz S, Park YS, Zengler K, Palsson BO. Deciphering the transcriptional regulatory logic of amino acid metabolism. Nat Chem Biol 2012; 8(1): 65-71.

Received: January 30, 2014 Revised: March 19, 2014 Accepted: April 03, 2014