Bacterial Swarming as a Protective Response to Intestinal Stress

Weijie Chen[1, 2, *], Arpan De[1, *], Hao Li[1, *], Dana J. Lukin[3], Wendy Szymczak[4], Katherine Sun[5], Libusha Kelly[6], Justin R. Wright[7], Regina Lamendella[8], Subho Ghosh[1], Daniel B. Kearns[9], Zhen He[10, †], Christian Jobin[10], Xiaoping Luo[1, ‡], Arjun Byju[1], Shirshendu Chatterjee[11], Beng San Yeoh[12, §], Matam Vijay-Kumar[12, §], Jay X. Tang[2], Sridhar Mani[1, **]

Affiliations:

[1]Department of Medicine, Genetics and Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA;

[2]Department of Physics, Brown University, 182 Hope Street, Providence, RI 02912, USA;

[3]Jill Roberts Center for Inflammatory Bowel Disease, 1283 York Avenue, New York, NY 10065, USA;

[4]Clinical Microbiology Laboratory, Montefiore Medical Center, 111 E 210th Street, Bronx, NY 10467, USA;

[5]Department of Pathology, NYU Langone Health, 560 First Avenue, New York, NY 10016, USA;

[6]Department of Systems & Computational Biology, and Department of Microbiology & Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA;

[7]Wright Labs, LLC, 419 14th Street, Huntington, PA 16652, USA;

[8]Juniata College, 1700 Moore Street, Huntingdon, PA 16652, USA;

[9]Department of Biology, Indiana University Bloomington, 107 S. Indiana Avenue, Bloomington, IN 47405, USA;

[10]Department of Medicine, University of Florida, Gainesville, FL 32611, USA;

[11]Department of Mathematics, The City College of New York, 160 Convent Avenue, New York, NY 10031, USA;

[12]UT-Microbiome Consortium, Department of Physiology & Pharmacology, University of Toledo, College of Medicine & Life Sciences, 3000 Transverse Dr, Mail Stop 1008, Toledo, OH 43614, USA

* Equal contribution ** Corresponding author

Correspondence to: Sridhar Mani, MD; **E-mail: sridhar.mani@einstein.yu.edu

† Present address: Department of Colorectal Surgery, The Sixth Affiliated Hospital of Sun Yat-Sen University, 26 Yuancun Erheng Road, Tianhe district, Guangzhou, Guangdong, China

‡ Present address: Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China

§ Present address: Department of Physiology and Pharmacology, University of Toledo, College of Medicine and Life Sciences, Toledo, OH 43614, USA

Abstract:

Bacterial swarming is a conserved and distinct form of bacterial motility that is often oppositely regulated and antagonistic to biofilm formation(*1*). To-date, while bacterial biofilms have been associated with pathogenesis and pathobiology of human diseases(*2-4*), there are very few examples of swarming behaviors that uniquely define or align with human pathophysiology(*5-7*). Here we report that swarming bacteria protects against intestinal inflammation in a murine model of colitis. Using feces in soft-agar plate assay we showed bacterial spreading harboring swarmers is highly predictive of the presence of intestinal stress in mice, pigs and humans. From murine feces, we isolated a novel *Enterobacter* swarming strain, SM3, which demonstrated significant protection from intestinal inflammation and promoted restitution in DSS-induced colitic mice. Known commensal swarmers also protected against intestinal inflammation when compared to swarming deficient isogenic mutants. Mechanistically, SM3 significantly reduced luminal oxygen concentration in colitic mice leading to a favorable anaerobic environment conducive to the growth of beneficial anaerobes. This work identifies a new paradigm in which intestinal stress, specifically inflammation, allows for emergence of swarming bacteria, which in turn has the ability to protect and heal from intestinal inflammation.

One Sentence Summary:

Bacterial swarming reduce intestinal inflammation.

Main Text:

Swarming, driven by flagella, is a fundamental process in certain groups of bacteria characterized by collective and rapid movement across a surface(*8, 9*). This process offers bacteria a competitive advantage in occupying certain niches (e.g., seeding colonization)(*10*); however, the cost-benefits to bacteria(*11, 12*) and consequences to its host or the environment remain largely unknown(*7*). Here we show that bacterial swarming is a hallmark of a stressed intestine. When swarming bacteria are present in sufficient abundance, the act of swarming *per se* suppresses intestinal stress in mammals. We posit that this occurs via the creation of a conducive anaerobic environment that leads to induction of beneficial anaerobes, which are associated with mucosal healing.

To test whether bacterial swarming is associated with human and rodent gut health, we developed a modified swarming assay using feces based on an established soft-agar plate assay utilized for single species(*13*). Since prototypical swarming bacteria (e.g., *Proteus mirabilis*, *Pseudomonas aeruginosa*) are associated with virulence(*7, 14*), we surmised that bacterial swarming might be well represented in colonoscopy samples and feces from humans with bacterial virulence associated pathologies (e.g., intestinal inflammation)(*15*). Colonoscopy aspirates were obtained from individuals with an active illness (inflammatory bowel disease - Crohn's and ulcerative colitis and other common forms of intestinal stress like intestinal polyps(*16, 17*) as well as age and gender matched controls (those without a clinically active illness). Within our sampling pool, bacterial collective spreading on soft agar was over-represented in cases with overt or clinically active intestinal stress (Fig. S1a-b). As a preliminary assessment, the presence of bacterial swarmers in feces was judged by the bacterial spread with a surfactant layer on soft-agar followed by isolation, identification by MALDI-TOF and validation of its swarming motility (Table S1). Nevertheless, this approach might have precluded selection of swarmers that do not produce surfactant(*8*). In

this pilot evaluation, the specificity and positive predictive value of the test for disease as defined was approximately, 88 and 89 %, respectively, while the sensitivity and negative predictive value of the test was only approximately 56 and 52 %, respectively (Fig. S1c). Similarly, feces collected from pigs with active inflammatory bowel disease also showed an increased prevalence of collective spreading and swarming as compared to control pigs (Fig. S1d). Together, these pilot data indicate that collective spreading and swarming is a specific feature, and potentially a biomarker of an intestinal pathology as defined by harboring active intestinal inflammation or polyps.

To identify the relevance of swarmers on host health, we focused on isolating endogenous swarming bacteria residing in rodents and humans. An initial approach was to determine if a singular dominant swarming species could always be isolated from a polymicrobial culture (such as mammalian feces). In a competitive swarming assay a mix of different pure bacterial cultures gave rise to a single bacterial species populating the leading edge of the swarm colony on agar (Fig. S2a-b). Similarly, swarming assays using pooled mouse or individual human feces yielded single species of a dominant swarmer as identified by MALDI-TOF (Table S1; Fig. S1e). To test whether swarming bacteria are also present in preclinical models, we screened feces of mice exposed to DSS, a chemical colitogen causing acute colonic inflammation(*18*, *19*). In a single experiment, we found three identical isolates from two different mouse fecal specimens- Strain 1 from mice exposed to water and, Strain 2 and 3 from mice exposed to dextran sulphate sodium (DSS), respectively (Fig. 1a). Swarming (in feces) was uniformly absent in vehicle exposed mice (Fig. S1e). The edge of the swarm colonies (as marked on Fig. 1a) were picked, serially passaged twice on 1% agar from a single colony and subsequently re-tested for swarm behavior on 0.5% agar plates (Fig. 1b). Strain 3 swarmed significantly faster compared to Strain 1 and 2.

Interestingly, 16S rRNA gene analysis and Multi Locus Sequence Typing (Fig. 1c) identified the isolated strains to be closest to *Enterobacter asburiae*. A whole genome sequence comparison of these *Enterobacter* strains (Fig. 1d) with related taxa *Enterobacter asburiae* and *Enterobacter cloacae*, revealed that all the three strains isolated here were "nearly identical" (>99% identical, Supplementary Discussion) and phylogenetically distinct from the reference strains. Taken together, using an agar-based assay to isolate dominant swarmers from a heterogenous culture, we were able to isolate nearly identical strains with striking difference in their swarming potential. Strain 1 (*Enterobacter* sp. SM1) originated from feces of vehicle (water) treated mice, while strain 2 (*Enterobacter* sp. SM2) and strain 3 (*Enterobacter* sp. SM3) originated from feces of DSS-induced colitic mice. Interestingly, a quantitative PCR sequencing-based approach to specifically identify SM1 or SM3 like bacteria in feces showed increase in its abundance during the evolution of DSS-induced colitis. The proportion of mice with high copy number values (>10,000 DNA copy/µL PCR reaction) was significantly higher in DSS group than water only group (Fig. S1f).

To determine the functional consequence of bacterial swarming in the host, DSS-induced colitic mice were administered the "near identical" swarming competent SM1 or SM3 strains. Both strains possess same growth rate and swim speed; however, unlike SM1, SM3 is a hyperswarmer (Fig. S3a-e; Supplementary Video 1). In contrast to that observed with SM1, SM3 significantly protected mice from intestinal inflammation (Fig. 2a-f). Specifically, SM3 significantly protected from body weight loss (Fig. 2a), increased colon length (Fig. 2b), reduced the colonic inflammation score (Fig. 2d), and had reduced expression of pro-inflammatory mediators compared to vehicle treated colitic mice (Fig. 2e-f). To test the mucosal healing capacity of swarming bacteria, we administered strains SM1 and SM3 to mice during the recovery phase of DSS exposure(*20*). When compared to vehicle, SM3 significantly improved weight gain and colon

length with reduced total inflammation and fibrosis at microscopic level (Fig. S4). To compare strict isogenic strains that only differed in swarming potential but not growth rate, surfactant production, or swimming speed (Fig. S3f-j), SM3 and isogenic mutants (SM3_18 and SM3_24) were administered to mice exposed to DSS. SM3, but not the swarming deficient mutants (SM3_18 and SM3_24), showed significant protection against weight loss, colon length and inflammation (Fig. 2g-i). Together, these data indicated that swarming *per se* is necessary for anti-inflammatory activity by SM3.

To determine if the anti-inflammatory role of SM3 is dependent on the conventional intestinal microbiome composition, germ-free mice transferred to specific pathogen free conditions (GF/SPF) and exposed to DSS-induced colitis, were treated with SM3. This strain was unable to protect against intestinal inflammation in GF/SPF mice (Fig. 3a). Fecal samples of colitic mice (conventional and GF/SPF) with SM3 administered were sent for microbiota analysis using 16S rRNA gene profiling. In contrast to GF/SPF mice, conventional mice feces showed specific enrichment of anaerobes belonging to the family S24-7 and Lactobacillaceae within SM3 treated mice when compared to vehicle mice (Fig. 3b). Specifically in conventional mice, we found significant increase in the abundance of S24-7 with SM3 gavage compared to vehicle in DSS exposed mice (Fig. 3c). SM3 does not affect the microbiota of non DSS treated mice, and the levels of S24-7 bacteria remains stable in SM3 treated group compared with untreated group (Fig. 3c). Within DSS exposed conventional mice, we observed that enriched S24-7 negatively co-occured with pathogenic taxa such as the Peptostreptococcaceae and Enterobacteriaceae (Fig. 3d).

The enrichment of certain specific anaerobes when treated with SM3 suggests a reduction in oxygen content in the intestine, however, during inflammation the median oxygen concentration increases (Fig. S5b). Swarming behavior of SM3 is dependent on oxygen concentration (Fig. S5a),

which in turn creates a reduced immediate environment at a significantly higher rate than the slow swarming variants (Fig. S5d). We hypothesized that swarming activity of SM3 may also reduce luminal oxygen concentrations *in vivo*. We determined the oxygen concentrations within the intestinal lumen of mice at various lengths along the colon. In control conventional C57BL/6 mice, the colonic lumen is uniformly "hypoxic and/or anoxic"; however, in colitic mice, we found a significant increase in the oxygen levels (ppm) in the colonic lumen (measured at different lengths from 0.5 to 2 cm proximal to the anal verge) (Fig. S5b). SM3 significantly reduced oxygen concentration compared to controls (vehicle); SM1 and the swarming deficient mutant strains did not significantly affect oxygen concentrations compared with controls (Fig. S5c). These results show that SM3, a hyperswarmer relative to SM1, but not swarming deficient strains or less dominant swarmers (i.e. SM1), consume oxygen rapidly. This may likely suggest that swarming activity *in vivo* reduces the oxygen content in the colonic lumen, which would aid in establishing an anaerobic (micro)environment.

To generalize this concept across multiple strains, mice with DSS induced colitis were administered *B. subtilis* 3610 (wildtype)(*21*) or its swarming deficient *swr*A isogenic mutant DS215(*22*) using the identical protocol as that used for SM3. In comparison with strain DS215, the wildtype significantly protected mice from intestinal inflammation (Fig.4a-e). Similarly, swarming *Serratia marcescens* Db10, in contrast to the swarming deficient JESM267 isogenic mutant, protected against inflammation in the identical mouse model (Fig. 4f-h). Incidentally, a clinical strain of *S. marcescens* (isolated from the surface washing of a human dysplastic polyp) also protected against DSS induced inflammation in mice (Supplementary Discussion). Similar to SM3, the swarming strains of *Bacillus* and *Serratia* deplete oxygen significantly faster than the isogenic non-swarming strains (Fig. S5e-f). These data suggest that a common mechanism might exist among swarmers, in that, via depletion of local oxygen concentrations they all induce a

favorable anaerobic environment. In addition, considering that the intestinal mucosa is relatively uneven during inflammation due to loss of mucin(*23*), we conjectured that swarmers may have an added advantage in niche dominance on inflamed tissue. Indeed, a mucosal race assay (Supplementary Discussion) showed that swarming bacteria finds advantage in motility on a colitic mucosa compared to normal mucosa (Fig. S6, Supplementary Video 2-4).

Together these studies demonstrate that intestinal inflammation promotes a niche conducive for bacterial swarming. The inflammatory milieu provides a permissive environment for stress adaptation and swarming behavior. Provided sufficient colonies form during inflammation, swarming strains deplete luminal oxygen content and allow for the intestine to re-establish conditions conducive to the growth of beneficial anaerobes. Consequently, swarming behavior could in turn suppress host inflammation by re-establishing homeostatic anaerobiosis in the gut (Fig. S7). Furthermore, our studies demonstrate the potential for a new personalized "probiotic" approach stemming from the ability to isolate and bank swarming microbes during colitis flares. These could be stored and provided back to the same individuals to prevent colitic episodes or as a therapeutic during acute colitis. In summary, our work demonstrates the unique and unprecedented role that bacterial swarming plays in intestinal homeostasis and in the potential clinical treatment of inflammatory bowel diseases.

References and Notes:

1. N. Verstraeten *et al.*, Living on a surface: swarming and biofilm formation. *Trends Microbiol* **16**, 496-506 (2008).
2. C. M. Dejea *et al.*, Microbiota organization is a distinct feature of proximal colorectal cancers. *Proc Natl Acad Sci U S A* **111**, 18321-18326 (2014).
3. M. T. Villanueva, Metabolism: Bacterial biofilms may feed colon cancer. *Nat Rev Cancer* **15**, 320 (2015).
4. C. H. Johnson *et al.*, Metabolism links bacterial biofilms and colon carcinogenesis. *Cell Metab* **21**, 891-897 (2015).
5. E. B. Breidenstein, C. de la Fuente-Nunez, R. E. Hancock, Pseudomonas aeruginosa: all roads lead to resistance. *Trends Microbiol* **19**, 419-426 (2011).
6. M. T. Butler, Q. Wang, R. M. Harshey, Cell density and mobility protect swarming bacteria against antibiotics. *Proc Natl Acad Sci U S A* **107**, 3776-3781 (2010).
7. C. Allison, L. Emody, N. Coleman, C. Hughes, The role of swarm cell differentiation and multicellular migration in the uropathogenicity of Proteus mirabilis. *J Infect Dis* **169**, 1155-1158 (1994).
8. D. B. Kearns, A field guide to bacterial swarming motility. *Nat Rev Microbiol* **8**, 634-644 (2010).
9. A. Be'er, G. Ariel, A statistical physics view of swarming bacteria. *Mov Ecol* **7**, 9 (2019).
10. J. D. Barak, L. Gorski, A. S. Liang, K. E. Narm, Previously uncharacterized Salmonella enterica genes required for swarming play a role in seedling colonization. *Microbiology* **155**, 3701-3709 (2009).
11. M. T. Butler, Q. Wang, R. M. Harshey, Cell density and mobility protect swarming bacteria against antibiotics. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 3776-3781 (2010).
12. A. Finkelshtein, D. Roth, E. Ben Jacob, C. J. Ingham, Bacterial Swarms Recruit Cargo Bacteria To Pave the Way in Toxic Environments. *mBio* **6**, (2015).
13. N. Morales-Soto *et al.*, Preparation, imaging, and quantification of bacterial surface motility assays. *J Vis Exp*, (2015).
14. J. Overhage, M. Bains, M. D. Brazas, R. E. Hancock, Swarming of Pseudomonas aeruginosa is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *J Bacteriol* **190**, 2671-2679 (2008).
15. Y. Yang, C. Jobin, Microbial imbalance and intestinal pathologies: connections and contributions. *Dis Model Mech* **7**, 1131-1142 (2014).
16. J. R. Jass, Hyperplastic-like polyps as precursors of microsatellite-unstable colorectal cancer. *Am J Clin Pathol.* **119**, 773-775 (2003).
17. J. Crespo-Sanjuán *et al.*, Early detection of high oxidative activity in patients with adenomatous intestinal polyps and colorectal adenocarcinoma: myeloperoxidase and oxidized low-density lipoprotein in serum as new markers of oxidative stress in colorectal cancer. *Lab Med.* **46**, 123-135 (2015).
18. M. Perse, A. Cerar, Dextran sodium sulphate colitis mouse model: traps and tricks. *J Biomed Biotechnol* **2012**, 718617 (2012).

19. B. Chassaing, J. D. Aitken, M. Malleshappa, M. Vijay-Kumar, Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr Protoc Immunol* **104**, Unit 15 25 (2014).
20. K. Suzuki *et al.*, Pivotal Role of Carbohydrate Sulfotransferase 15 in Fibrosis and Mucosal Healing in Mouse Colitis. *PLoS One* **11**, e0158967 (2016).
21. D. B. Kearns, R. Losick, Swarming motility in undomesticated Bacillus subtilis. *Mol Microbiol* **49**, 581-590 (2003).
22. D. B. Kearns, F. Chu, R. Rudner, R. Losick, Genes governing swarming in Bacillus subtilis and evidence for a phase variation mechanism controlling surface motility. *Mol Microbiol* **52**, 357-369 (2004).
23. Y. Sasaki, S. Fukuda, T. Mikam, R. Hada, Endoscopic quantification of mucosal surface roughness for grading severity of ulcerative colitis. *Digestive Endoscopy* **20**, 67-72 (2008).
24. T. Seemann, Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068-2069 (2014).
25. A. E. Darling, B. Mau, N. T. Perna, progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* **5**, e11147 (2010).
26. S. H. Yoon, S. M. Ha, J. Lim, S. Kwon, J. Chun, A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* **110**, 1281-1286 (2017).
27. A. Be'er, S. K. Strain, R. A. Hernandez, E. Ben-Jacob, E. L. Florin, Periodic reversals in Paenibacillus dendritiformis swarming. *J Bacteriol* **195**, 2709-2717 (2013).
28. A. Sokolov, I. S. Aranson, Reduction of viscosity in suspension of swimming bacteria. *Phys Rev Lett* **103**, 148101 (2009).
29. H. P. Zhang, A. Be'er, E. L. Florin, H. L. Swinney, Collective motion and density fluctuations in bacterial colonies. *Proc Natl Acad Sci U S A* **107**, 13626-13630 (2010).
30. T. E. Angelini, M. Roper, R. Kolter, D. A. Weitz, M. P. Brenner, Bacillus subtilis spreads by surfing on waves of surfactant. *Proc Natl Acad Sci U S A* **106**, 18109-18113 (2009).
31. Y. Y. Lee, J. Patellis, R. Belas, Activity of Proteus mirabilis FliL is viscosity dependent and requires extragenic DNA. *J Bacteriol* **195**, 823-832 (2013).
32. E. B. Steager, C. B. Kim, M. J. Kim, Dynamics of pattern formation in bacterial swarms. *Physics of Fluids* **20**, (2008).
33. A. E. Patteson, A. Gopinath, P. E. Arratia, The propagation of active-passive interfaces in bacterial swarms. *Nat Commun* **9**, 5373 (2018).
34. R. Selvam *et al.*, Effect of Bacillus subtilis PB6, a natural probiotic on colon mucosal inflammation and plasma cytokines levels in inflammatory bowel disease. *Indian J Biochem Biophys* **46**, 79-85 (2009).
35. D. M. Newsom, G. L. Bolgos, L. Colby, J. A. Nemzek, Comparison of body surface temperature measurement and conventional methods for measuring temperature in the mouse. *Contemp Top Lab Anim Sci* **43**, 13-18 (2004).
36. A. A. Bodour, R. M. Miller-Maier, Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *Journal of Microbiological Methods* **32**, 273-280 (1998).
37. B. A. Dilmohamud, J. Seeneevassen, S. D. D. V. Rughooputh, P. Ramasami, Surface tension and related thermodynamic parameters of alcohols using the Traube stalagmometer. *European Journal of Physics* **26**, 1079-1084 (2005).

38. M. Koster, W. van Klompenburg, W. Bitter, J. Leong, P. Weisbeek, Role for the outer membrane ferric siderophore receptor PupB in signal transduction across the bacterial cell envelope. *EMBO J* **13**, 2805-2813 (1994).
39. L. Burbank, M. Mohammadi, M. C. Roper, Siderophore-Mediated Iron Acquisition Influences Motility and Is Required for Full Virulence of the Xylem-Dwelling Bacterial Phytopathogen Pantoea stewartii subsp stewartii. *Applied and Environmental Microbiology* **81**, 139-148 (2015).
40. M. J. McBride, Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu Rev Microbiol* **55**, 49-75 (2001).
41. B. M. Schultz *et al.*, Persistent Salmonella enterica serovar Typhimurium Infection Increases the Susceptibility of Mice to Develop Intestinal Inflammation. *Front Immunol* **9**, 1166 (2018).
42. U. Attmannspacher, B. E. Scharf, R. M. Harshey, FliL is essential for swarming: motor rotation in absence of FliL fractures the flagellar rod in swarmer cells of Salmonella enterica. *Mol Microbiol* **68**, 328-341 (2008).
43. R. Belas, R. Suvanasuthi, The ability of Proteus mirabilis to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein. *J Bacteriol* **187**, 6789-6803 (2005).
44. I. Lagkouvardos *et al.*, Sequence and cultivation study of Muribaculaceae reveals novel species, host preference, and functional potential of this yet undescribed family. *Microbiome* **7**, 28 (2019).
45. M. A. Borton *et al.*, Chemical and pathogen-induced inflammation disrupt the murine intestinal microbiome. *Microbiome* **5**, 47 (2017).
46. T. Osaka *et al.*, Meta-Analysis of Fecal Microbiota and Metabolites in Experimental Colitic Mice during the Inflammatory and Healing Phases. *Nutrients* **9**, (2017).
47. S. P. Colgan, C. T. Taylor, Hypoxia: an alarm signal during intestinal inflammation. *Nat Rev Gastroenterol Hepatol* **7**, 281-287 (2010).
48. K. L. Ormerod *et al.*, Genomic characterization of the uncultured Bacteroidales family S24-7 inhabiting the guts of homeothermic animals. *Microbiome* **4**, 36 (2016).
49. T. Okada *et al.*, Microbiota-derived lactate accelerates colon epithelial cell turnover in starvation-refed mice. *Nat Commun* **4**, 1654 (2013).
50. E. R. Hughes *et al.*, Microbial Respiration and Formate Oxidation as Metabolic Signatures of Inflammation-Associated Dysbiosis. *Cell Host Microbe* **21**, 208-219 (2017).
51. M. E. Johansson, H. Sjovall, G. C. Hansson, The gastrointestinal mucus system in health and disease. *Nat Rev Gastroenterol Hepatol* **10**, 352-361 (2013).
52. E. Sun, S. J. Liu, R. E. W. Hancock, Surfing Motility: a Conserved yet Diverse Adaptation among Motile Bacteria. *Journal of Bacteriology* **200**, (2018).
53. H. Sies, Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol* **11**, 613-619 (2017).
54. P. M. Munyaka, S. Sepehri, J. E. Ghia, E. Khafipour, Carrageenan Gum and Adherent Invasive Escherichia coli in a Piglet Model of Inflammatory Bowel Disease: Impact on Intestinal Mucosa-associated Microbiota. *Frontiers in microbiology* **7**, 462 (2016).
55. D. H. Huson *et al.*, A simple statistical test of taxonomic or functional homogeneity using replicated microbiome sequencing samples. *J Biotechnol* **250**, 45-50 (2017).

56. D. B. Kearns, R. Losick, Cell population heterogeneity during growth of Bacillus subtilis. *Genes Dev* **19**, 3083-3094 (2005).
57. M. Venkatesh *et al.*, Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* **41**, 296-310 (2014).
58. C. G. Whittem, A. D. Williams, C. S. Williams, Murine Colitis modeling using Dextran Sulfate Sodium (DSS). *J Vis Exp*, (2010).
59. U. Erben *et al.*, A guide to histomorphological evaluation of intestinal inflammation in mouse models. *Int J Clin Exp Pathol* **7**, 4557-4576 (2014).
60. J. McCafferty *et al.*, Stochastic changes over time and not founder effects drive cage effects in microbial community assembly in a mouse model. *ISME J* **7**, 2116-2125 (2013).
61. T. J. Wiles *et al.*, Combining quantitative genetic footprinting and trait enrichment analysis to identify fitness determinants of a bacterial pathogen. *PLoS Genet* **9**, e1003716 (2013).
62. J. T. Saavedra, J. A. Schwartzman, M. S. Gilmore, Mapping Transposon Insertions in Bacterial Genomes by Arbitrarily Primed PCR. *Curr Protoc Mol Biol* **118**, 15 15 11-15 15 15 (2017).
63. W. Walters *et al.*, Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems* **1**, (2015).
64. R. C. Edgar, Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460-2461 (2010).
65. J. G. Caporaso *et al.*, QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**, 335-336 (2010).
66. J. G. Caporaso *et al.*, Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* **108 Suppl 1**, 4516-4522 (2011).
67. R. C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* **10**, 996-998 (2013).
68. T. Z. DeSantis *et al.*, Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* **72**, 5069-5072 (2006).
69. N. Segata *et al.*, Metagenomic biomarker discovery and explanation. *Genome Biol* **12**, R60 (2011).
70. P. Shannon *et al.*, Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**, 2498-2504 (2003).
71. K. Faust, J. Raes, CoNet app: inference of biological association networks using Cytoscape. *F1000Res* **5**, 1519 (2016).
72. W. Kim, M. G. Surette, Prevalence of surface swarming behavior in Salmonella. *J Bacteriol* **187**, 6580-6583 (2005).
73. A. Bankevich *et al.*, SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* **19**, 455-477 (2012).
74. P. Yilmaz *et al.*, The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res* **42**, D643-648 (2014).
75. M. Alanjary, K. Steinke, N. Ziemert, AutoMLST: an automated web server for generating multi-locus species trees highlighting natural product potential. *Nucleic Acids Res* **47**, W276-W282 (2019).

76. N. F. Alikhan, N. K. Petty, N. L. Ben Zakour, S. A. Beatson, BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* **12**, 402 (2011).
77. P. C. Lau, C. K. Sung, J. H. Lee, D. A. Morrison, D. G. Cvitkovitch, PCR ligation mutagenesis in transformable streptococci: application and efficiency. *J Microbiol Methods* **49**, 193-205 (2002).
78. S. Datta, N. Costantino, D. L. Court, A set of recombineering plasmids for gram-negative bacteria. *Gene* **379**, 109-115 (2006).
79. T. A. Hooven *et al.*, The essential genome of Streptococcus agalactiae. *BMC Genomics* **17**, 406 (2016).
80. P. P. Cherepanov, W. Wackernagel, Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* **158**, 9-14 (1995).
81. V. Walter, C. Syldatk, R. Hausmann, Screening Concepts for the Isolation of Biosurfactant Producing Microorganisms. *Adv Exp Med Biol* **672**, 1-13 (2010).
82. B. Coburn, Y. Li, D. Owen, B. A. Vallance, B. B. Finlay, Salmonella enterica serovar Typhimurium pathogenicity island 2 is necessary for complete virulence in a mouse model of infectious enterocolitis. *Infect Immun* **73**, 3219-3227 (2005).
83. R. Geesala *et al.*, Loss of RHBDF2 results in an early-onset spontaneous murine colitis. *J Leukoc Biol* **105**, 767-781 (2019).
84. D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting Linear Mixed-Effects Models Using lme4. *2015* **67**, 48 (2015).
85. A. Kuznetsova, P. B. Brockhoff, R. H. B. Christensen, lmerTest Package: Tests in Linear Mixed Effects Models. *2017* **82**, 26 (2017).
86. J. Fox, *Applied regression analysis and generalized linear models, 2nd ed*. Applied regression analysis and generalized linear models, 2nd ed. (Sage Publications, Inc, Thousand Oaks, CA, US, 2008), pp. xxi, 665-xxi, 665.
87. J. Fox, S. Weisberg, J. Fox, *An R companion to applied regression*. (2011).
88. J. P. Royston, Algorithm AS 181: The W Test for Normality. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **31**, 176-180 (1982).
89. J. P. Royston, An Extension of Shapiro and Wilk's W Test for Normality to Large Samples. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **31**, 115-124 (1982).
90. P. Royston, Remark AS R94: A Remark on Algorithm AS 181: The W-test for Normality. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **44**, 547-551 (1995).
91. W. Dong, Y. K. Matsuno, A. Kameyama, A procedure for Alcian blue staining of mucins on polyvinylidene difluoride membranes. *Anal Chem* **84**, 8461-8466 (2012).
92. K. Lee, E. Tosti, W. Edelmann, Mouse models of DNA mismatch repair in cancer research. *DNA Repair* **38**, 140-146 (2016).

Acknowledgements: We thank Steve Almo, Cait Costello, Jeffrey Pessin, Matthew R. Redinboand John March for valuable discussions. We aslo thank Brad Tricomi for developing the assay "Cytotoxicity of DSS on Caco-2 cell lines in the presence or absence of viable SM3 cells", Ehsan Khafipour for providing pig specimens (feces) and performing clinical scoring of histopathology, Cori Bargmann at Rockfeller University for gifting us the bacterial strains *Serratia marcescens* Db10 and *Serratia marcescens* JESM267, and Barry Bochner at Biolog Inc., California for Biochemical characterization and antimicrobial resistance profile of *Enterobacter* sp. SM1, SM2 and SM3. Additional invaluable assistance was obtained from Amanda Beck DVM (Histology and Comparative Pathology Core, Albert Einstein College of Medicine, Bronx, NY), Olga C. Arionadis, Thomas Ullmann and Azal Al Ani (Department of Medicine, Albert Einstein College of Medicine, Bronx, NY), Winfred Edelmann and Eleni Tosti (Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY).

Funding: The studies presented here were supported in part by the Broad Medical Research Program at CCFA (Crohn's & Colitis Foundation of America; Grant# 362520) (to S.M); NIH R01 CA127231; CA 161879; 1R01ES030197-01 and Department of Defense Partnering PI (W81XWH-17-1-0479; PR160167) (S.M.), Diabetes Research Center Grant (P30 DK020541); Cancer Center Grant (P30CA013330 PI: David Goldman); 1S10OD019961-01 NIH Instrument Award (PI: John Condeelis); LTQ Orbitrap Velos Mass Spectrometer System (1S10RR029398); and NIH CTSA (1 UL1 TR001073). Peer Reviewed Cancer Research Program Career Development Award from the United States Department of Defense (CA171019, PI: Libusha Kelly).

Author Contributions: H.L., S.M. conceptualized the discovery. H.L., D.K., W.C., J.T., S.M. designed and executed the swarming assays. D.L. was the Principal Investigator of the Clinical Study and provided specimens. L.K. performed genome assembly and annotation. J.W., R.L., S.M. designed and executed all the 16S, metagenomic and strain-specific PCR assays. A.D. designed; A.D., W.C., S.M., S.G. characterized bacterial mutants. B.S.Y. and M.V-K. performed several swarming repeat assays and performed animal studies for reproducibility. A.D., H.L., W.C. and S.M. wrote and edited the paper. S.C. and W.C. performed statistical analyses. X.L. assisted H.L. in mouse model studies. A.B. analyzed the clinical data and revised the paper. K.S. did the histological preparations and examination. C.J. and Z.H. performed gnotobiotic mouse model studies. W.S. identified bacteria strains using MALDI-TOF.

Competing Financial Interests: Sridhar Mani, Libusha Kelly, and Hao Li filed a U.S. patent application (Application No. 62237657). Other authors declare no competing financial interests.

Data and materials availability: All data is available in the main text or the supplementary materials.

Supplementary Materials:

Supplementary Text

Materials and Methods

Figures S1-S17

Tables S1-S5

Supplementary video 1-6

References (24-*92*)

Figure 1

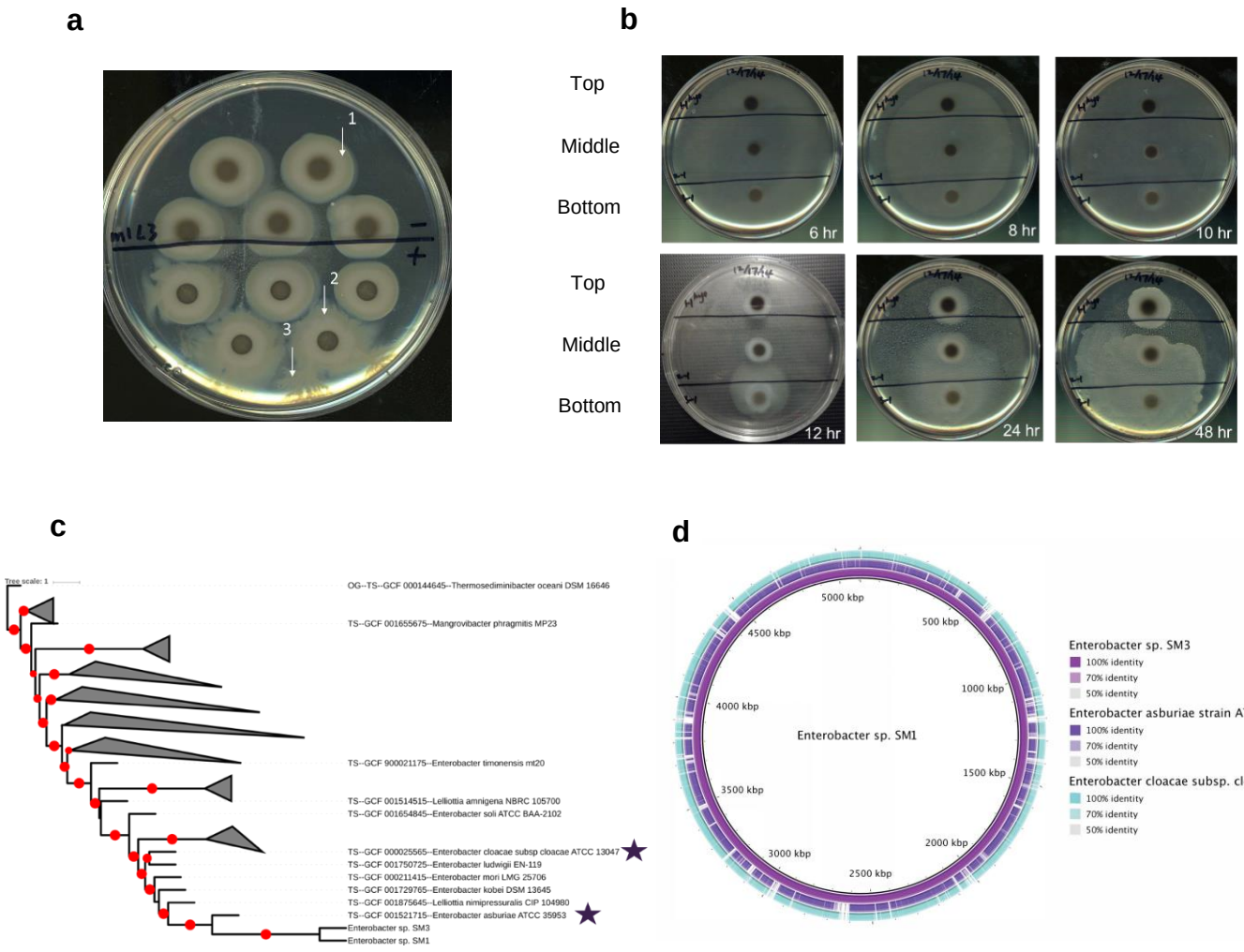

Figure 1 | Isolation and characterization of *Enterobacter* sp. a, Five replicate fecal spots from pooled fecal pellets of mice administered water (above black line) or 3% DSS water (below black line) (n = 3, day 7). The white arrows indicate 1, swarm edge isolation from control feces (SM1); 2, swarm edge isolation from feces of mice exposed to DSS (SM2); 3, swarm colony isolation from spontaneous "burst" activity from feces at 24h from plating (SM3). The mouse experiments were repeated at least twice. **b,** The bacterial clones isolated from **a** were re-plated as pure strains on 0.5% LB agar and the swarming assay performed over time. Two solid black marker lines divide each plate into 3 regions, holding spots of the 3 strains – Top: Strain 1 (SM1), Middle: Strain 2 (SM2), Bottom: Strain 3 (SM3). These strains have been repeatedly (≥25 times) plated in swarming assays from all aliquots stored from the original isolation (August 2014) and the results confirm that SM3 is a stable hyperswarmer. **c**, Phylogenetic tree showing multi-locus sequencing typing-based genetic relatedness between *Enterobacter* sp. SM1, SM3 and reference genomes. Tree was generated with autoMLST (CITE) and drawn using iTOL (CITE). Red dots indicate bootstrap support > 0.8. Stars represent related strains used for comparison with the genome sequences of SM1 and SM3 in panel **d**. **d,** Genome comparison of related *Enterobacter* strains. *Enterobacter*sp. SM1 was compared to *Enterobacter*sp. SM3 (purple) and the related strains *Enterobacter asburiae* ATCC 35953 (violet) and *Enterobacter cloacae* ATCC 13047 (cyan), and plotted in BLAST Ring Generator (BRIG) http://brig.sourceforge.net/ PMID: 21824423. DSS, Dextran Sulfate Sodium; LB, Luria-Bertani broth.

Figure 2

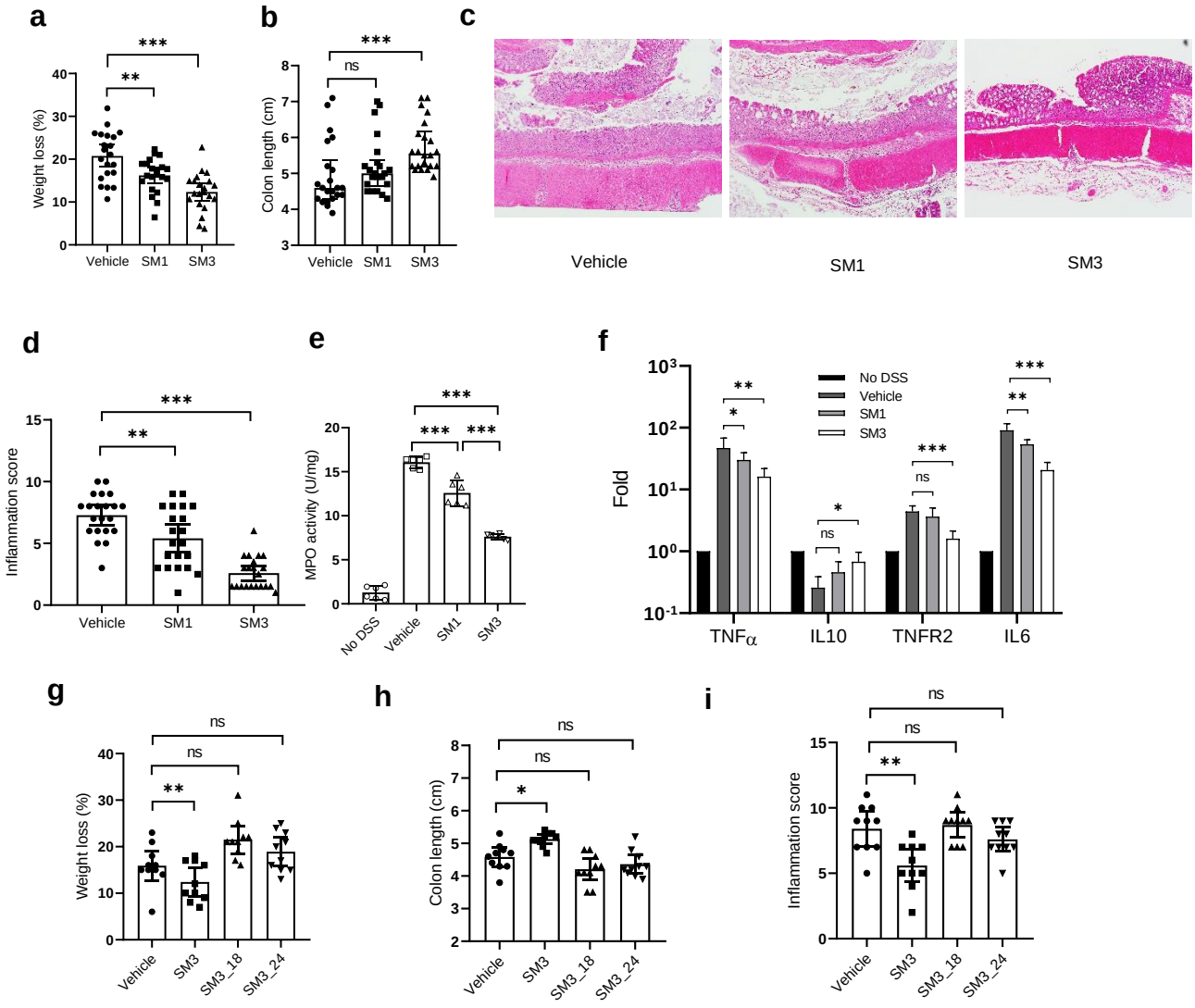

Figure 2 | Effects of *Enterobacter* sp. SM strains on DSS induced colitis in C57BL/6 mice. a-f, 8-weekold mice were exposed to DSS water and treated with vehicle (LB), SM1 or SM3 by oral gavage for 10 days. **a-b** indicates weight loss (**a**) and colon length (**b**) (n = 21 per treatment group). **c**, Representative images (100x magnification) of H&E stained colonic section treated with vehicle (left), SM1 (middle) and SM3 (right). **d,** Inflammation score (n = 21 per treatment group). **e-f,** In a separate experiment, myeloperoxidase (MPO) enzyme activity was determined (n= 3, each in duplicate) (**e**). Colon total RNA (n = 4) was isolated and reverse transcribed to cDNA. RT-qPCR data show fold induction of mRNA (TNFα, IL10, TNFR2, IL6). PCR was repeated in quadruplicate. The expression was normalized to internal control, TBP. The entire experiment was repeated n = 2 for reproducibility (**f**).**g-i,** In a separate experiment, C57BL/6 mice (8-week old) were exposed to DSS water and administered vehicle (LB), SM3, or its mutants (SM3_18 or SM3_24) for 10 days. **g-i** indicates weight loss (**g**), colon length (**h**) and inflammation score (**i**) (n = 10 per treatment group). Unless otherwise noted, data are represented as mean and 95% CI, and significance tested using one-way ANOVA followed by Tukey's post hoc test. **c,** data represented as median and interquartile range, and significance tested using Kruskal-Wallis followed by Dunn's multiple comparisons test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant. H&E, Hematoxylin and Eosin; TBP, TATA-Box Binding Protein; CI, Confidence Interval.

Figure 3

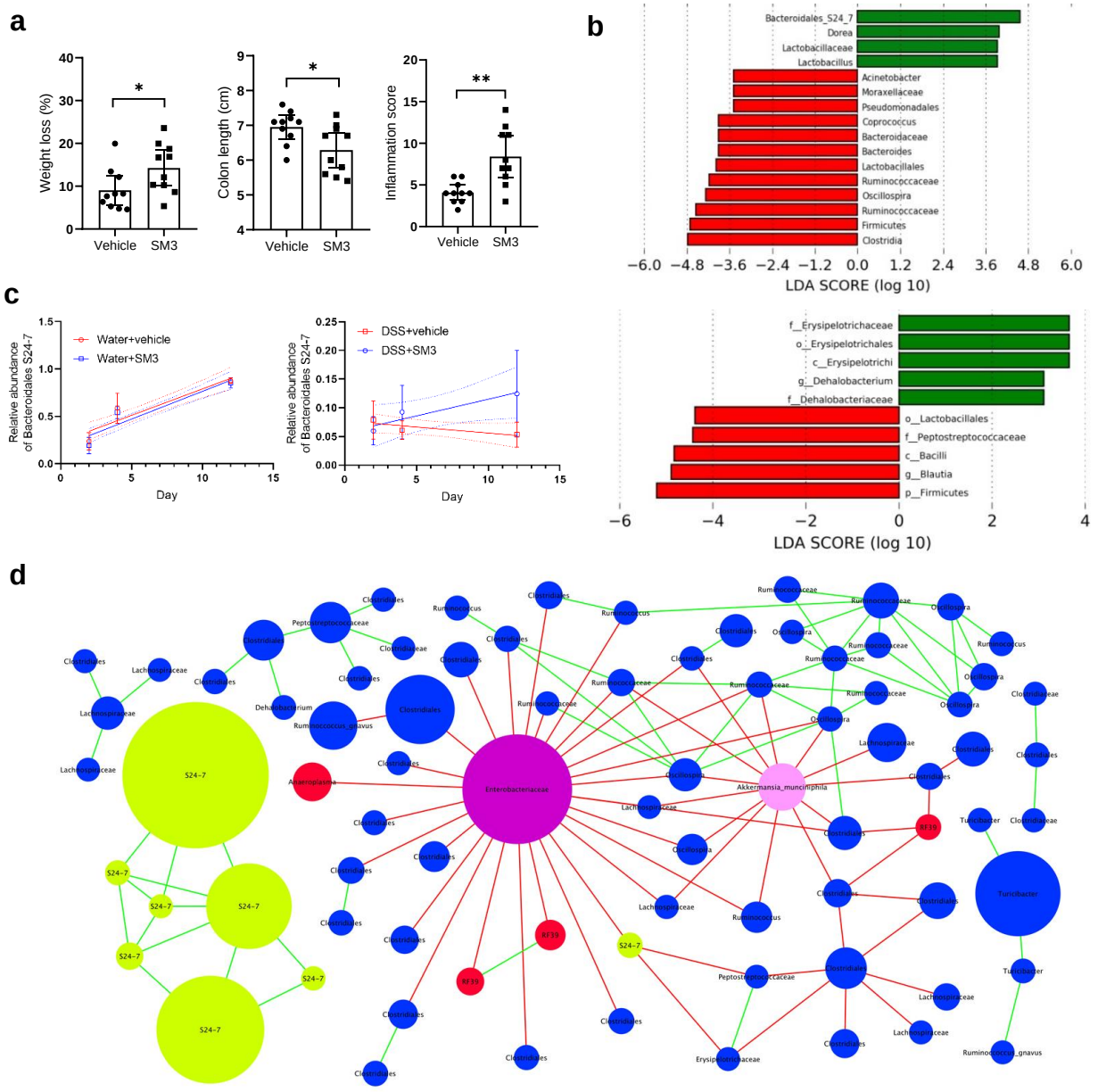

Figure 3 | Effects of SM3 on the intestinal microbiota of GF/SPF and conventional mice. a, C57BL/6 GF/SPF mice (5-week old) were exposed to DSS water and treated with vehicle (LB) or SM3 for 6 days. **a** indicates weight loss (left), colon length (middle), and inflammation score (right) (n = 10 per treatment group). **b,** Linear discriminant analysis (LDA) Effect Size (LEfSe) plot of taxonomic biomarkers identified using feces of SM3 treated conventional (n = 10) (upper) and GF/SPF (n = 10) (lower) colitic mice on day 12 and day 6, respectively, as compared to vehicle (n = 10). Green and red bars indicate bacterial enrichment within SM3 treated and vehicle group respectively. All taxa that yielded an LDA score >3.0 are presented. **c,** Relative abundance of S24-7 in the feces from DSS (right) and control (left) mice treated with SM3 or vehicle (n = 8 per treatment group). Linear regression line was fit to show the trend of the change (dotted lines show the 95% confidence bands). The slope of the SM3 treated group is similar to vehicle in water control group (P = 0.7827), but significantly different in DSS group (P = 0.0182). **d,** Co-occurrence network plot showing strong positive and negative correlations (Spearman's $|\rho| > 0.7$) between OTU abundances. Each node represents a single OTU and the size of each node is proportional to the relative abundance of each OTU. Green lines connecting two nodes indicate a strong positive correlation (spearman's $\rho > 0.7$) between the taxa, whereas red lines indicate a strong negative correlation (spearman's $\rho < -0.7$) between the taxa. Unless otherwise noted, data are represented as mean and 95% CI, and significance tested using a two-tailed Student's t-test. OTU, Operational Taxonomic Unit; GF/SPF, Germ-Free mice transferred to specific pathogen free conditions.

Figure 4

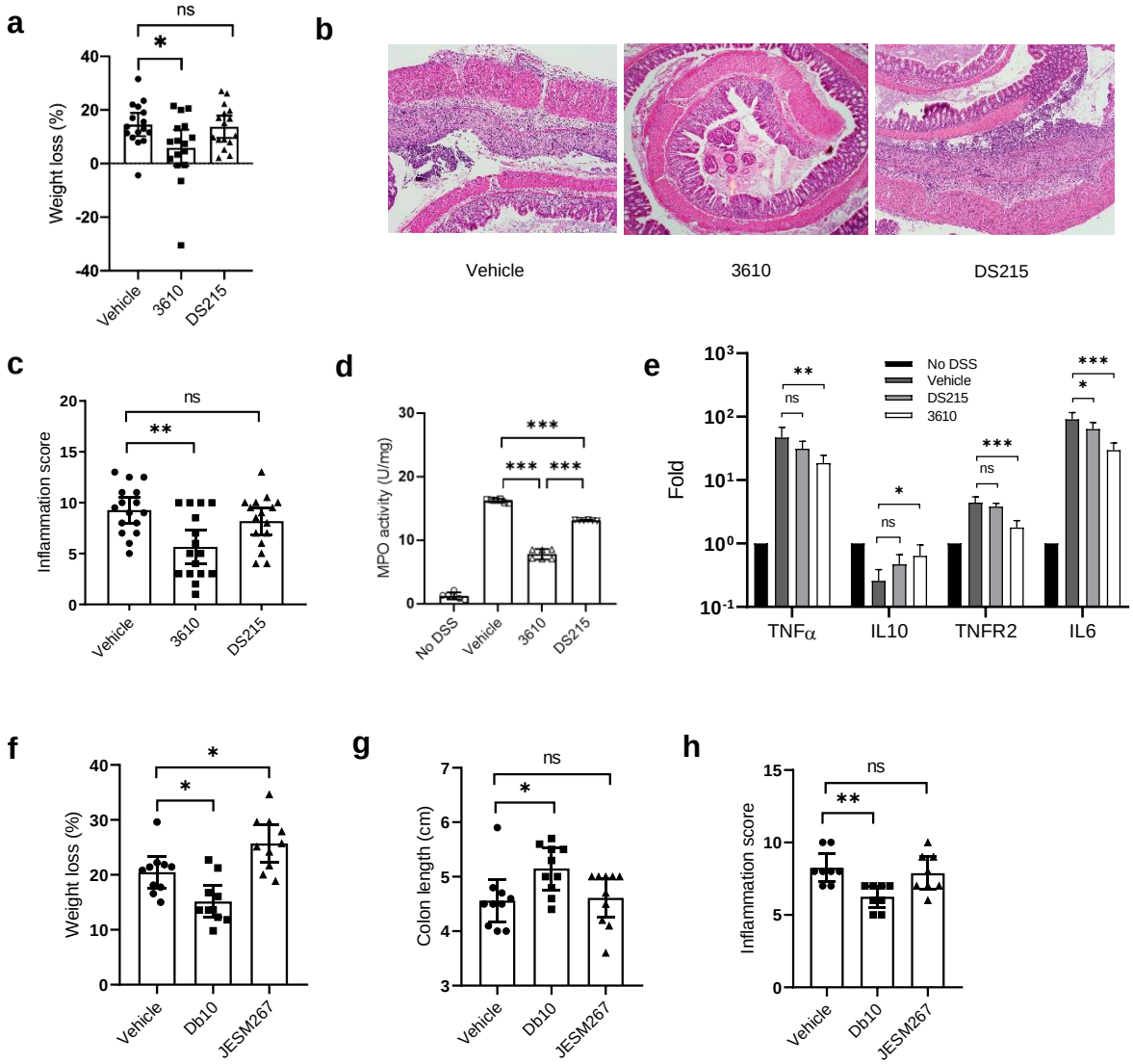

Figure 4 | Effects of *B. subtilis* and *S. marcescens* on DSS induced colitis in C57BL/6 mice. a-e, 8-week old mice were exposed to DSS water and treated with vehicle (LB), *B. subtilis* 3610 or *B. subtilis* DS215 by oral gavage for 10 days. **a,** Weight loss (n = 16 per treatment group). **b,** Representative images (100x magnification) of H&E stained colonic section treated with vehicle (left), 3610 (middle) and DS215 (right). **c,** Inflammation score (n = 16 per treatment group). **d-e,** In a separate experiment, myeloperoxidase (MPO) enzyme activity was determined (n = 3, each in duplicate) (**d**). Colon total RNA (n = 4) were isolated and reverse transcribed to cDNA. RT-qPCR data show fold induction of mRNA (TNFα, IL10, TNFR2, IL6). PCR was repeated in quadruplicate. The expression was normalized to internal control, TBP. The entire experiment was repeated n = 2 for reproducibility (**e**). **f-h,** In a separate experiment, C57BL/6 mice (8-week old) were exposed to DSS water and administered vehicle (LB), *S. marcescens* Db10 or *S. marcescens* JESM267 for 10 days. **f-h** indicates weight loss (**f**), colon length (**g**) and inflammation score (**h**) (n = 10 per treatment group except for **h**, for which n = 8; two colon specimens per group were used for other experiments). Unless otherwise noted, data represented as mean and 95% CI, and significance tested using one-way ANOVA followed by Tukey's post hoc test. **g,** data represented as median and interquartile range, and significance tested using Kruskal-Wallis followed by Dunn's multiple comparisons test.