


Genome Sequence of a *Proteus mirabilis* Strain Isolated from the Salivary Glands of Larval *Lucilia sericata*

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We announce a draft genome sequence of a *Proteus mirabilis* strain derived from *Lucilia sericata* salivary glands. This strain is demonstrated to attract and induce oviposition by *L. sericata*, a common blow fly important to medicine, agriculture, and forensics. The genome sequence will help dissect interkingdom communication between the species.

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Proteus mirabilis is a gut-commensal bacterium associated with human urinary tract infections (1–4) and is a model for cellular communication (5–8). It is found in association with rotting proteinaceous material (9–13), the blow fly *Lucilia sericata* (14) (a fly used in maggot therapy [15]), and other flies associated with decomposing animal remains and animal wounds (9, 13). There are several reasons to hypothesize a commensal relationship between these species. *P. mirabilis* is hypothesized to enhance maggot therapy (15). This enhancement is partially due to the production of antibiotic molecules (16, 17), which kill microbes that are effectively controlled in maggot therapy (15, 18, 19). This aligned microbial control suggests that the bacterium and fly are in competition with similar bacterial species. Concurrently, the flies do not appear to effectively control *P. mirabilis* (19). Further, *Proteus* species have been identified in salivary gland samples of *L. sericata* (14, 20), a relatively clean tissue and a major source of molecules contributing to molecular antibacterial activities important to maggot therapy (21–24). Finally, swarming signals associated with *P. mirabilis* have been linked to fly attraction and oviposition, making the species a model for interkingdom signaling between bacteria and insects (7), which might have implications for medical, forensic, and agricultural research with decomposer flies and for microbial ecology.

Here, we present a draft genomic sequence of *P. mirabilis*. Genomic DNA was isolated from a colony derived from maggot salivary glands of *L. sericata* third-instar larvae raised on beef liver (7). Sequencing was performed using an Ion Torrent Personal Genome Machine (Life Technologies, Carlsbad, CA) after preparation with a NEBNext fast DNA fragmentation library prep set. This produced approximately 1,880,512 short reads, with an average length of 219 bp, totaling 412 Mbp, resulting in approximately 104-fold coverage. A total of 113 contigs were assembled using the PATRIC assembly service (25), with an N_{50} of 202,584 bp. This strain is highly similar to previously sequenced *P. mirabilis* HI4320 (NCBI accession no. NC_010554) and

BB2000 (NCBI accession NC_022000), being more similar to BB2000. Draft genome assemblies based on CONTIGuator (26) indicate 49 contigs unique to this strain, with 98.6% of the assembled nucleotides aligning to either of the reference genomes. These observations support a previous finding that strains from this species exhibit lineage specific indels (27, 28), suggesting a species with a core genome and various auxiliary genes. Two contigs were found to have plasmid identities of >99%.

The draft genome contigs consist of 3,953,708 bp, with 38.43% G+C content. A total of 3,678 genes and 3,586 coding sequences (CDSs) were identified by the NCBI Prokaryotic Genome Annotation Pipeline (29). Seven prophage regions were identified among contigs with PHAST (30), of which three regions are intact, three are incomplete, and one is questionably functional. One of the prophage sequences predicted to be active is located near *rfaL*, which has been shown to impact fly behavior (7). Strain-specific gene functions and phage insertions will be useful in dissecting the interactions between *L. sericata* and *P. mirabilis*.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [LTBK000000000](https://www.ncbi.nlm.nih.gov/nuclink/LTBK000000000); this is version [LTBK010000000](https://www.ncbi.nlm.nih.gov/nuclink/LTBK010000000).

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REFERENCES

- Allison C, Coleman N, Jones PL, Hughes C. 1992. Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Infect Immun* 60:4740–4746.
- Umpiérrez A, Scavone P, Romanin D, Marqués JM, Chabalgoity JA, Rumbo M, Zunino P. 2013. Innate immune responses to *Proteus mirabilis* flagellin in the urinary tract. *Microbes Infect* 15:688–696. <http://dx.doi.org/10.1016/j.micinf.2013.06.007>.
- Mobley HL, Belas R. 1995. Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. *Trends Microbiol* 3:280–284. [http://dx.doi.org/10.1016/S0966-842X\(00\)88945-3](http://dx.doi.org/10.1016/S0966-842X(00)88945-3).
- Coker C, Poore CA, Li X, Mobley HL. 2000. Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes Infect* 2:1497–1505. [http://dx.doi.org/10.1016/S1286-4579\(00\)01304-6](http://dx.doi.org/10.1016/S1286-4579(00)01304-6).
- Gibbs KA, Urbanowski ML, Greenberg EP. 2008. Genetic determinants of self identity and social recognition in bacteria. *Science* 321:256–259. <http://dx.doi.org/10.1126/science.1160033>.
- Stankowska D, Czerwonka G, Rozalska S, Grosicka M, Dziadek J, Kaca W. 2012. Influence of quorum sensing signal molecules on biofilm formation in *Proteus mirabilis* O18. *Folia Microbiol (Praha)* 57:53–60. <http://dx.doi.org/10.1007/s12223-011-0091-4>.
- Ma Q, Fonseca A, Liu W, Fields AT, Pimslar ML, Spindola AF, Tarone AM, Crippen TL, Tomberlin JK, Wood TK. 2012. *Proteus mirabilis* interkingdom swarming signals attract blow flies. *ISME J* 6:1356–1366. <http://dx.doi.org/10.1038/ismej.2011.210>.
- Dienes L. 1946. Reproductive processes in *Proteus* cultures. *Exp Biol Med* 63:265–270. <http://dx.doi.org/10.3181/00379727-63-15570>.
- Kamal AS. 1959. Comparative studies of thirteen species of Sarcosaprophagous *Calliphoridae* and *Sarcophagidae* (Diptera) II Digestive enzymology. *Ann Entomol Soc Am* 52:167–173. <http://dx.doi.org/10.1093/aesa/52.2.167>.
- Yoshinaga DH, Frank HA. 1982. Histamine-producing bacteria in decomposing skipjack tuna *Katsuwonus pelamis*. *Appl Environ Microbiol* 44:447–452.
- Durlu-Özkaya F, Ayhan K, Vural N. 2001. Biogenic amines produced by *Enterobacteriaceae* isolated from meat products. *Meat Sci* 58:163–166. [http://dx.doi.org/10.1016/S0309-1740\(00\)00144-3](http://dx.doi.org/10.1016/S0309-1740(00)00144-3).
- Lauber CL, Metcalf JL, Keepers K, Ackermann G, Carter DO, Knight R. 2014. Vertebrate decomposition is accelerated by soil microbes. *Appl Environ Microbiol* 80:4920–4929. <http://dx.doi.org/10.1128/AEM.00957-14>.
- Thompson CR, Brogan RS, Scheifele LZ, Rivers DB. 2013. Bacterial interactions with necrophagous flies. *Ann Entomol Soc Am* 106:799–809. <http://dx.doi.org/10.1603/AN12057>.
- Singh B, Crippen TL, Zheng L, Fields AT, Yu Z, Ma Q, Wood TK, Dowd SE, Flores M, Tomberlin JK, Tarone AM. 2015. A metagenomic assessment of the bacteria associated with *Lucilia sericata* and *Lucilia cuprina* (Diptera: Calliphoridae). *Appl Microbiol Biotechnol* 99:869–883. <http://dx.doi.org/10.1007/s00253-014-6115-7>.
- Sherman RA, Hall MJ, Thomas S. 2000. Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annu Rev Entomol* 45:55–81. <http://dx.doi.org/10.1146/annurev.ento.45.1.55>.
- Greenberg B. 1968. Model for destruction of bacteria in the midgut of blow fly maggots. *J Med Entomol* 5:31–38. <http://dx.doi.org/10.1093/jmedent/5.1.31>.
- Erdmann GR, Khalil SKW. 1986. Isolation and identification of two antibacterial agents produced by a strain of *Proteus mirabilis* isolated from larvae of the screwworm *Cochliomyia hominivorax* (Diptera: Calliphoridae). *J Med Entomol* 23:208–211. <http://dx.doi.org/10.1093/jmedent/23.2.208>.
- Čerovský V, Žďárek J, Fučík V, Monincová L, Voburka Z, Bém R. 2010. Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blowfly *Lucilia sericata*. *Cell Mol Life Sci* 67:455–466. <http://dx.doi.org/10.1007/s00018-009-0194-0>.
- Jaklič D, Lapanje A, Zupančič K, Smrke D, Gunde-Cimerman N. 2008. Selective antimicrobial activity of maggots against pathogenic bacteria. *J Med Microbiol* 57:617–625. <http://dx.doi.org/10.1099/jmm.0.047515-0>.
- Blenkiron C, Tsai P, Brown LA, Tintinger V, Askelund KJ, Windsor JA, Phillips AR. 2015. Characterisation of the small RNAs in the biomedically important green-bottle blowfly *Lucilia sericata*. *PLoS One* 10:e0122203. <http://dx.doi.org/10.1371/journal.pone.0122203>.
- Bexfield A, Nigam Y, Thomas S, Ratcliffe NA. 2004. Detection and partial characterisation of two antibacterial factors from the excretions/secretions of the medicinal maggot *Lucilia sericata* and their activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *Microbes Infect* 6:1297–1304. <http://dx.doi.org/10.1016/j.micinf.2004.08.011>.
- Bexfield A, Bond AE, Roberts EC, Dudley E, Nigam Y, Thomas S, Newton RP, Ratcliffe NA. 2008. The antibacterial activity against MRSA strains and other bacteria of a <500Da fraction from maggot excretions/secretions of *Lucilia sericata* (Diptera: Calliphoridae). *Microbes Infect* 10:325–333. <http://dx.doi.org/10.1016/j.micinf.2007.12.011>.
- Harris LG, Bexfield A, Nigam Y, Rohde H, Ratcliffe NA, Mack D. 2009. Disruption of *Staphylococcus epidermidis* biofilms by medicinal maggot *Lucilia sericata* excretions/secretions. *Int J Artif Organs* 32:555–564.
- Bexfield A, Bond AE, Morgan C, Wagstaff J, Newton RP, Ratcliffe NA, Dudley E, Nigam Y. 2010. Amino acid derivatives from *Lucilia sericata* excretions/secretions may contribute to the beneficial effects of maggot therapy via increased angiogenesis. *Br J Dermatol* 162:554–562. <http://dx.doi.org/10.1111/j.1365-2133.2009.09530.x>.
- Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res* 42:D581–D591. <http://dx.doi.org/10.1093/nar/gkt1099>.
- Galardini M, Biondi EG, Bazzicalupo M, Mengoni A. 2011. CONTIGuator: a bacterial genomes finishing tool for structural insights on draft genomes. *Source Code Biol Med* 6:11. <http://dx.doi.org/10.1186/1751-0473-6-11>.
- Sullivan NL, Septer AN, Fields AT, Wenren LM, Gibbs KA. 2013. The complete genome sequence of *Proteus mirabilis* strain BB2000 reveals differences from the *P. mirabilis* reference strain. *Genome Announc* 1(5):e00024–13. <http://dx.doi.org/10.1128/genomeA.00024-13>.
- Pearson MM, Sebahia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, Abdallah Z, Arrosmith C, Atkin B, Chillingworth T, Hauser H, Jagels K, Moule S, Mungall K, Norbertczak H, Rabinowitzsch E, Walker D, Whithead S, Thomson NR, Rather PN. 2008. Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. *J Bacteriol* 190:4027–4037. <http://dx.doi.org/10.1128/JB.01981-07>.
- Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyripides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of standard operating procedures (SOPs) for metagenomic annotation. *Omic* 12:137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
- Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res* 39:W347–W352. <http://dx.doi.org/10.1093/nar/gkr485>.