AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Draft Genome Sequences of Biosafety Level 2 Opportunistic Pathogens Isolated from the Environmental Surfaces of the International Space Station

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The draft genome sequences of 20 biosafety level 2 (BSL-2) opportunistic pathogens isolated from the environmental surfaces of the International Space Station (ISS) were presented. These genomic sequences will help in understanding the influence of microgravity on the pathogenicity and virulence of these strains when compared with Earth strains.

Received 20 September 2016 **Accepted** 31 October 2016 **Published** 29 December 2016

Citation Checinska Sielaff A, Singh NK, Allen JE, Thissen J, Jaing C, Venkateswaran K. 2016. Draft genome sequences of biosafety level 2 opportunistic pathogens isolated from the environmental surfaces of the International Space Station. Genome Announc 4(6):e01263-16. doi:10.1128/genomeA.01263-16.

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In an on-going Microbial Observatory experiment on the International Space Station (ISS), multiple biosafety level 2 (BSL-2) bacterial isolates were isolated, identified, and whole-genome sequences (WGS) were generated. The genomic data enables the determination of the microgravity influence on pathogenicity and virulence in these microorganisms by comparison to type strains of the corresponding species.

Acinetobacter pittii is a nonmotile coccobacilli isolated for the first time from cerebrospinal fluid (1). Multiple strains of *A. pitti* were isolated from the cupola area. The IIF1SW-P1 was resistant to cefazolin, cefoxitin, oxacillin, penicillin, and rifampin.

Two multidrug-resistant *Enterobacter* sp. isolates were found in the waste and hygiene compartment (WHC) location. Species of *Enterobacter cloacae* complex (Ecc) are commonly found in the environment, but are of high clinical significance (2).

TABLE 1 Statistics summary for the 20 draft ISS BSL-2 bacterial genome sequences

Strain	NCBI accession no.	Isolation location	No. of contigs	Genome size (bp)	N_{50} (bp)	Median coverage	G+C content (%)	Error corrected reads	Coding sequences
A. pittii IIF1SW-P1	MIZX00000000	Port panel next to cupola	150	4,041,255	144,373	799	38.7	25,486,884	3,821
Enterobacter sp. IF2SW-B1	MJAA00000000	WHC[a]	437	5,097,299	306,837	686	55.2	24,992,043	4,671
Enterobacter sp. IF2SW-P2	MJAB00000000	WHC[a]	230	4,974,814	298,912	850	55.8	30,618,796	4,629
P. conspicua IF5SW-P1	MIZY00000000	Node 1 overhead 4	280	5,126,609	216,776	797	55.6	34,104,170	4,852
S. aureus IF4SW-P1	MIZH00000000	Dining table	498	2,980,137	64,789	3,695	32.7	76,859,228	2,733
S. aureus IF6SW-P2	MIZI00000000	PMM port 1[b]	204	2,836,553	355,893	2,578	32.8	51,467,673	2,657
S. aureus IF6SW-P2-RA	MIZK00000000	PMM port 1[b]	228	2,845,178	295,897	2,740	32.8	55,167,977	2,659
S. aureus IF6SW-P3A	MIZJ00000000	PMM port 1[b]	276	2,868,506	232,680	2,254	32.8	46,555,897	2,694
S. aureus IF6SW-P3A-RA	MIZL00000000	PMM port 1[b]	257	2,861,821	264,865	2,733	32.8	47,711,605	2,690
S. aureus IF7SW-P3	MIZM00000000	Lab overhead 3	452	2,951,917	52,140	3,487	32.8	71,062,021	2,738
S. aureus IIF6SW-P2	MIZN00000000	PMM port 1[b]	312	2,884,460	96,689	3,324	32.8	67,792,619	2,730
S. aureus IIF6SW-P2-RA	MIZR00000000	PMM port 1[b]	192	2,835,299	325,968	2,021	32.8	42,250,883	2,655
S. aureus IIF6SW-P3	MIZO00000000	PMM port 1[b]	194	2,837,901	467,825	2,638	32.8	54,334,144	2,657
S. aureus IIF6SW-P3-RA	MIZS00000000	PMM port 1[b]	217	2,841,156	411,108	2,272	32.8	47,711,605	2,656
S. aureus IIF8SW-P1	MIZP00000000	Port crew quarters bump-out exterior aft wall	143	2,817,304	425,858	2,409	32.8	49,197,886	2,637
S. aureus IIF8SW-P1-RA	MIZT00000000	Port crew quarters bump-out exterior aft wall	201	2,848,005	526,364	1,834	32.7	39,316,061	2,653
S. aureus IIF8SW-P2	MIZQ00000000	Port crew quarters bump-out exterior aft wall	194	2,830,972	329,726	2,557	32.8	51,625,221	2,650
S. aureus IIF8SW-P2-RA	MIZU00000000	Port crew quarters bump-out exterior aft wall	141	2,822,756	526,364	2,014	32.8	42,704,251	2,642
S. haemolyticus IIF2SW-P5	MIZW00000000	WHC[a]	567	2,680,722	48,308	2,945	33.1	56,836,461	2,518
S. hominis IIF4SC-B9	MIZV00000000	Dining table	508	2,420,684	79,555	3,738	31.5	61,283,456	2,301

[a] WHC, waste and hygiene compartment.
[b] PMM port 1, permanent multipurpose module.

Pantoea conspicua was originally isolated from human blood (3). This was the second most prevalent species, and was only found in one location during two different flight samplings. *P. conspicua* isolates were resistant to erythromycin, oxacillin, penicillin, and rifampin.

Staphylococcus isolates were the most prevalent from ISS surfaces. *Staphylococcus aureus* was the most abundant in all ISS locations. Although this species is a common human commensal (4), it causes various types of minor skin infections, bacteremia, or scalded skin syndrome, especially in immunocompromised individuals (5). In this study, some of the isolates were found to be resistant to erythromycin (IF4SW-P1, IF7SW-P3) and most of the isolates were resistant to penicillin. A few isolates acquired rifampin resistance during the study (RA isolates).

Staphylococcus haemolyticus and *Staphylococcus hominis* belong to coagulase-negative staphylococci (6, 7). *S. hominis* IIF4SC-B9 was resistant to penicillin and erythromycin, but *S. haemolyticus* IIF2SW-P5 was susceptible to these antibiotics. All three species are reported to be methicillin resistant by acquiring the staphylococcal cassette chromosome *mec* (SCC*mec*) (8), but the methicillin-resistant phenotype was not observed.

In this study, the draft genomes sequences of 20 strains from the ISS were obtained. WGS sequencing was performed on an Illumina NextSeq instrument with a paired-end module. The A5 assembly pipeline version 20150522 was used to generate draft assemblies applying the default parameter settings (9) and annotated with the help of the Rapid Annotations using Subsystems Technology (RAST) (10). Table 1 summarizes assembly statistics (number of contigs, total genome size, N_{50} size, median coverage, G+C percentage, error corrected reads used for assembly, and number of coding sequences). The raw reads were in the range of 24 to 82 Mbp per genome. The G+C content was in the range of 31.5 to 38.7% for *Staphylococcus* species and *A. pittii*; for other strains the G+C contents were 55.2 to 55.8%. The subsystem features created using RAST for all 20 strains are depicted in Table 1.

Accession number(s). The WGS data were deposited at DDBL/EMBL/GenBank under the accession no. listed in Table 1 and at the NASA GeneLab system (GLDS-67; https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-67/##). The version described in this paper is the first version. The strains were deposited in the USDA Agricultural Research Station (NRRL) and German culture collections.

ACKNOWLEDGMENTS

We thank the implementation team (lead by Fathi Karouia) of the Microbial Observatory (Microbial Tracking) project at NASA Ames Research Center.

Part of the research described in this publication was carried out at the Jet Propulsion Laboratory, California Institute of Technology, and at the Lawrence Livermore National laboratory, under a contract with NASA.

FUNDING INFORMATION

This work, including the efforts of Aleksandra Checinska Sielaff, Nitin Kumar Singh, and Kasthuri Venkateswaran, was funded by National Aeronautics and Space Administration (NASA) (19-12829-26 and 19-12829-27). This work, including the efforts of Jonathan Allen, James B. Thissen, and Crystal Jaing, was funded by National Aeronautics and Space Administration (NASA) (NNX15AJ29G).

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