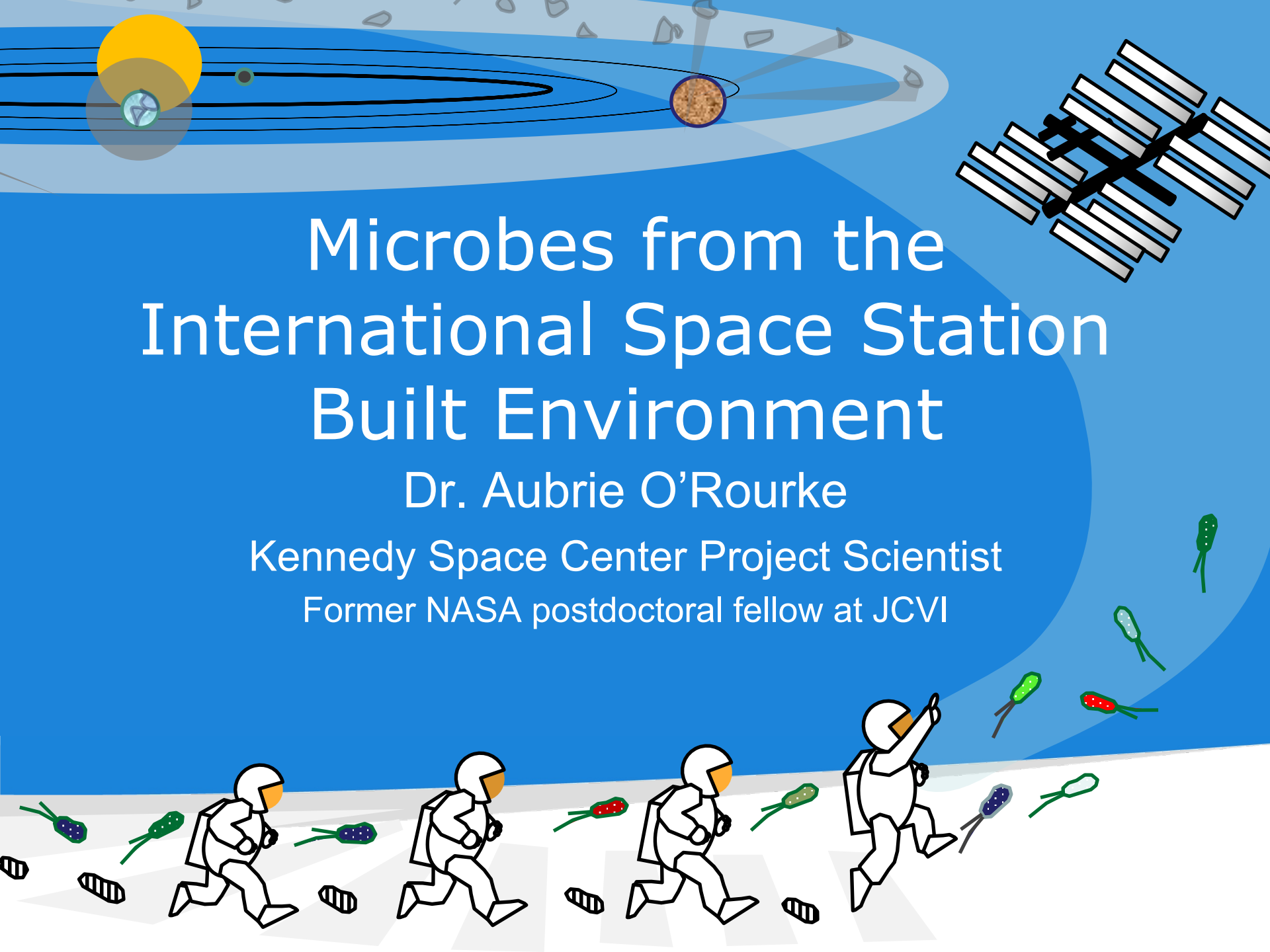


Microbes from the International Space Station Built Environment

Dr. Aubrie O'Rourke

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Former NASA postdoctoral fellow at JCVI

MICROBES OF THE BUILT ENVIRONMENT (MoBE)

This fellowship was made possible by the Space Act Agreement between NASA and Alfred P. Sloan Foundation's MoBE research initiative

MoBE premise and objectives:

- Given that a typical person spends ~90% of their time indoors, MoBE research aims to know more about the microbial diversity of this environment.
- Of particular interest is the prevalence of species affecting human health, including both opportunistic and primary pathogens.

MoBE terrestrial indoor environment findings:

- Indoor surfaces and dust are widely colonized by human-associated and environmental microorganisms introduced via direct contact and passive deposition from inhabitants, transported materials, and the air supply.

THE BUILT ENVIRONMENT

THE ISS AS A CASE STUDY

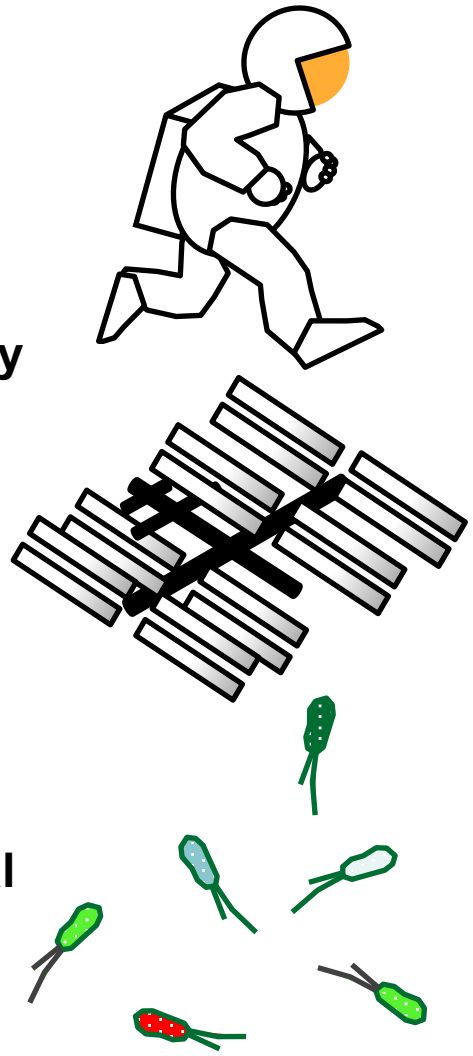
The **ISS** has **constant human occupancy**, **controlled environmental conditions** and **routine microbial monitoring** to ensure crew safety, spanning nearly two decades.

Viable members of the **ISS microbiome are presumably acclimated to selective pressures** such as

- low-nutrient, dry settings,
- microgravity, lowered shear force
- elevated CO2,
- and cosmic radiation.

Adaptive mechanisms are thought to include:

- **mobile genetic element** (MGE)-**mediated horizontal gene transfer** (HGT),
- Gene mutation,
- and DNA rearrangement or loss



PLANETARY PROTECTION

MICROBIAL SURVEILLANCE

Surveillance of the air, surfaces and water of the ISS

- **Uses standard culturing techniques**
- **Has been conducted over twelve years and 22 missions**
- **Resulted in a collection of 424 bacterial cultivates encompassing 34 genera**

***Staphylococcus* isolates account for 70% of those recovered from surface samples, and 80% of those recovered from air samples.**

***Burkholderia* and *Ralstonia* species together account for 60% of the bacterial isolates obtained from the potable water system of the International Space Station.**

WORKFLOW

CULTURE

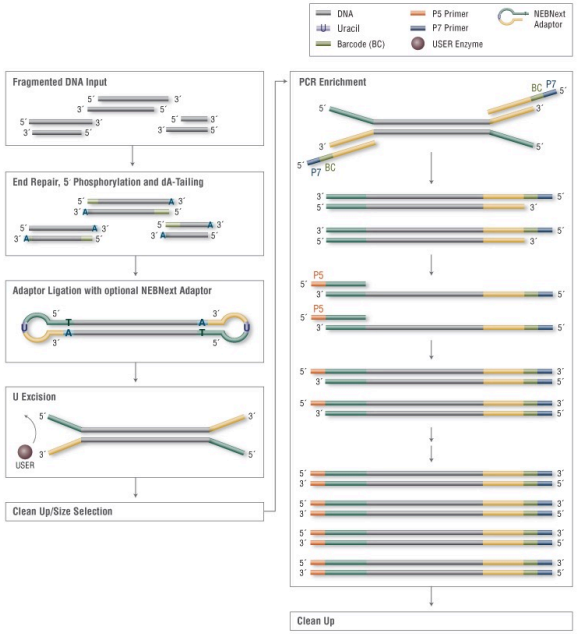
- **24 BURKHOLDERIA ISOLATES**
- **42 STAPHYLOCOCCUS ISOLATES**



PHENOL/CHOLOFORM HMW DNA EXTRACTION



LIBRARY PREPARATION



SEQUENCING



CORRELATE GENOTYPE TO PHENOTYPE

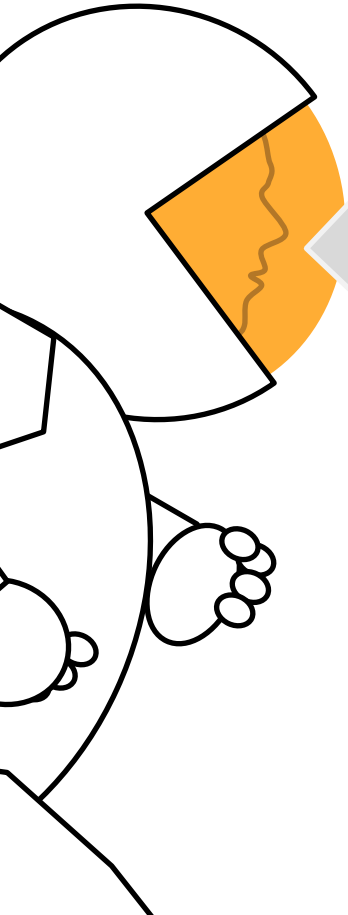
VARIOUS PHENOTYPIC ASSAYS TO ASSESS VIRULENCE AND ANTIBIOTIC DRUG RESISTANCE

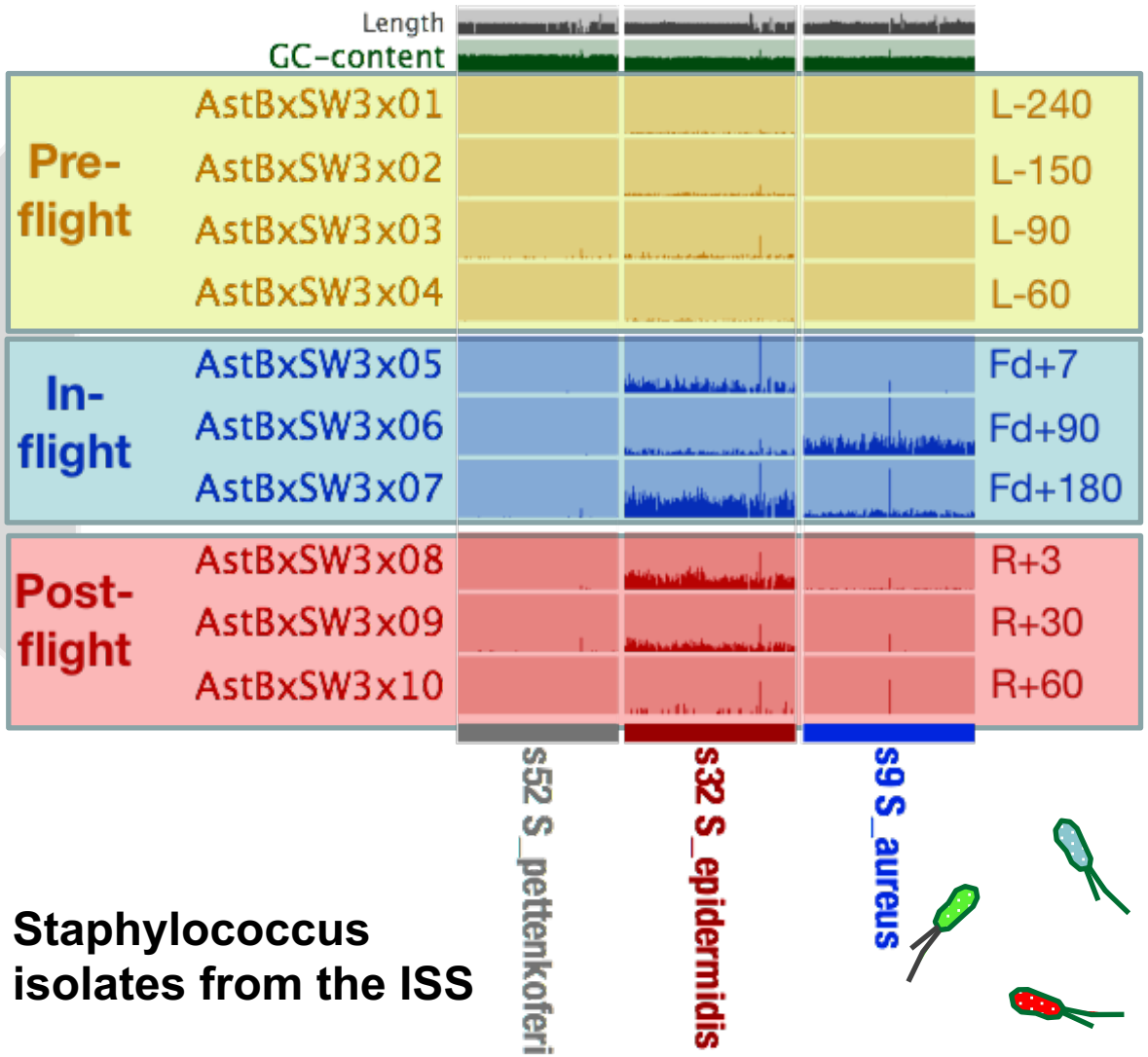
GENOTYPIC ANALYSIS OF ISOLATES

ASTRONAUTS AS VECTORS

Mapping astronaut microbiome to our isolates

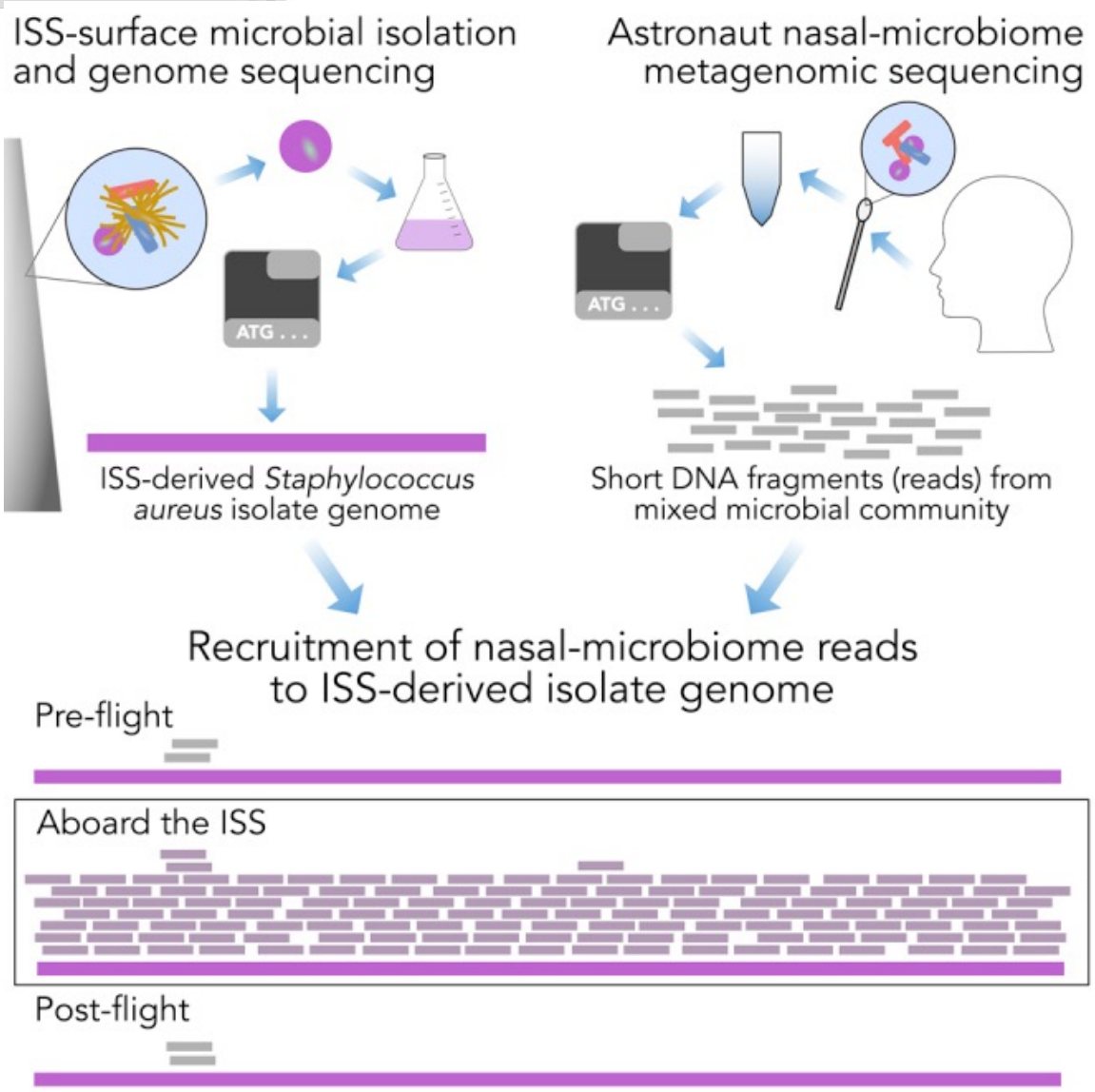
One astronaut's nasal microbiome samples through time





Staphylococcus isolates from the ISS

Read recruitment of astronaut metagenomic samples to ISS isolate genomes



STAPHYLOCCOUS EPIDERMIDIS

***Staphylococcus* sp. are associated with the healthy-skin microbiome**

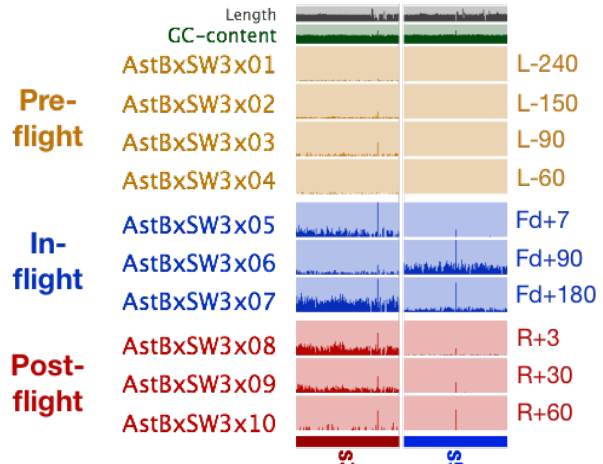
- Specific *Staphylococcus* can produce antimicrobial peptides (AMP) that are selectively antagonistic to *S. aureus*
  - Some protect against *S. aureus* in a murine model
  - can alleviate existing *S. aureus* colonization in patients with atopic dermatitis

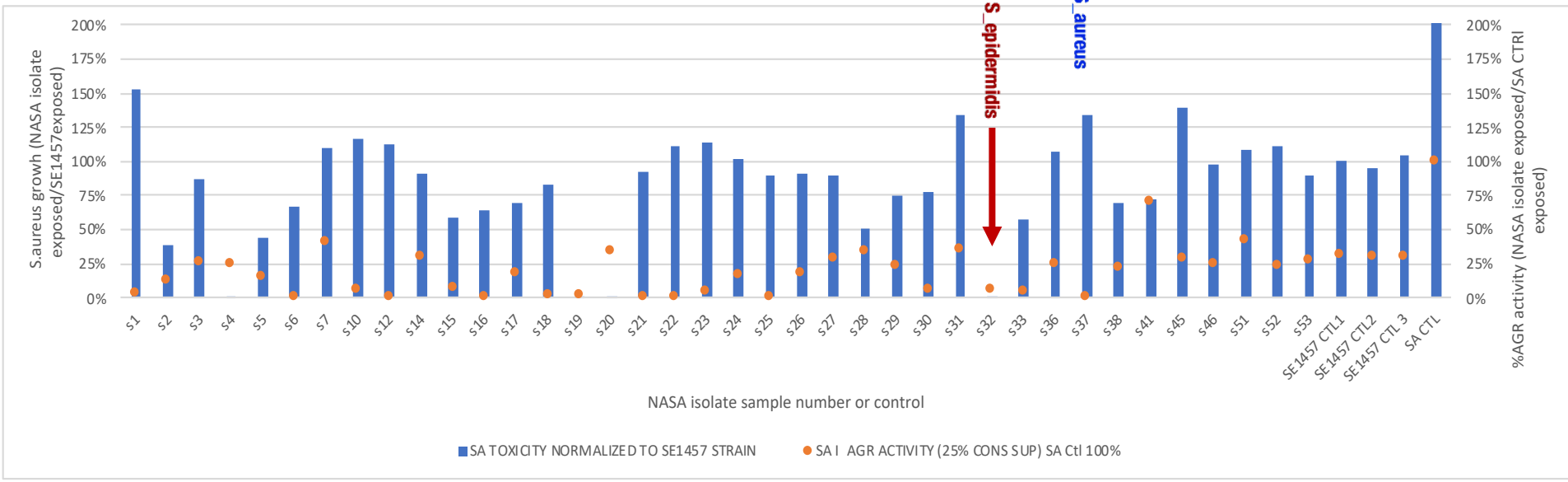
***S. epidermidis* can also exhibit virulence**

- Extracellular cysteine proteases (Ecp) have been associated with the colonization and infection of human tissues.

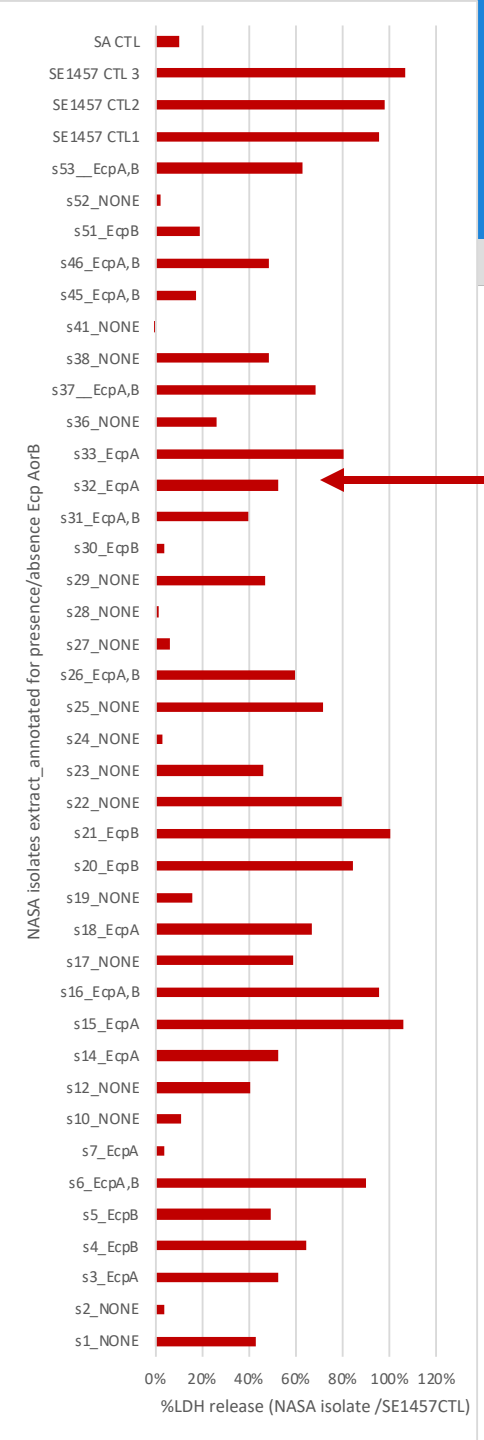
S. EPIDERMIDIS CAN INHIBIT GROWTH OF S. AUREUS

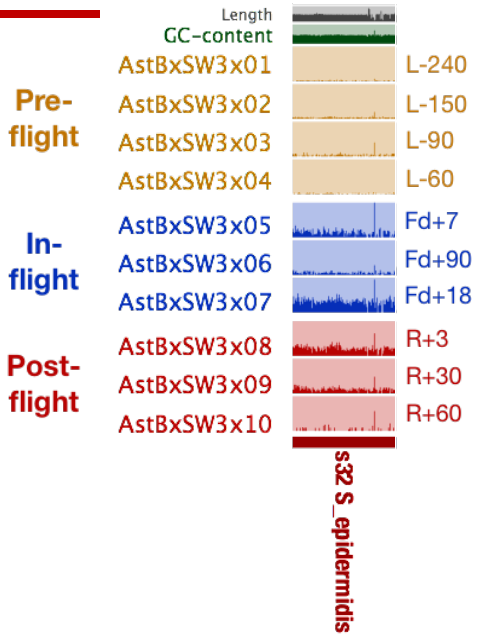
ISS *S. epidermidis* isolate #s32 both inhibits *S. aureus* growth and *S. aureus* quorum sensing ability





S. EPIDERMIDIS ISOLATES CAN LYSE KERATINOCYTES





ISS *S. epidermidis* isolate #s32 lyses keratinocytes at a rate that is half that of the virulent terrestrial control strain SE1457

S32 also harbors the EcpA virulence-associated toxin.

ISS S. EPIDERMIDIS SUMMARY

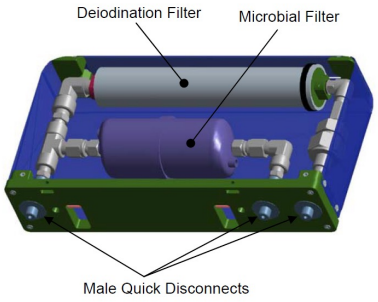
ISS *S. epidermidis* isolate **s32** likely **originated from an astronaut then spread to the ISS surface**.

This isolate has the **ability to inhibit *S. aureus* growth** and quorum sensing mechanisms.

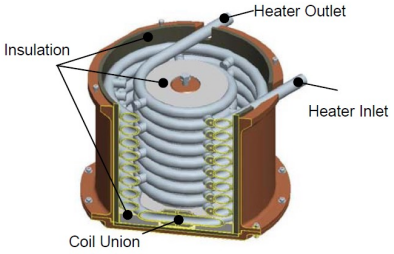
The isolate likely harbors **the ability (half that of the terrestrial control) to lyse keratinocytes**, perhaps due to the presence of EcpA.

BURKHOLDERIA OF THE POTABLE WATER SYSTEM

Burkholderia cepacia and ***Burkholderia contaminans***- disease-causing genomovars of the *B. cepacia* complex (BCC):



- **Can survive long periods in distilled water**
  - ISS *Burkholderia* isolates were collected over a span of six years (2009-2015) during Missions 20-45 to the ISS.
- **Can survive as biofilms within an infected individual.**
  - Cystic fibrosis patients with a chronic BCC colonization live on average 5.2 years after infection.
  - Immunocompromised individuals are also susceptible to infection.



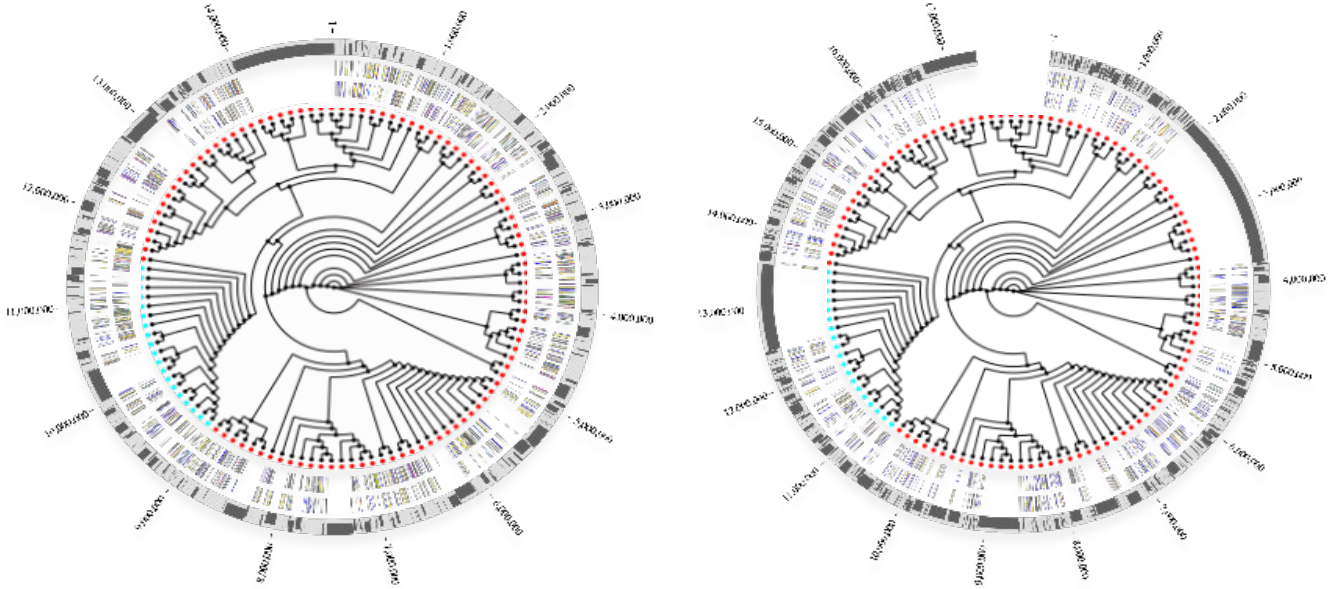
- ***Burkholderia*** **have mobile genetic elements (MGEs), that promote acquisition of virulence genes and antibiotic resistance**.



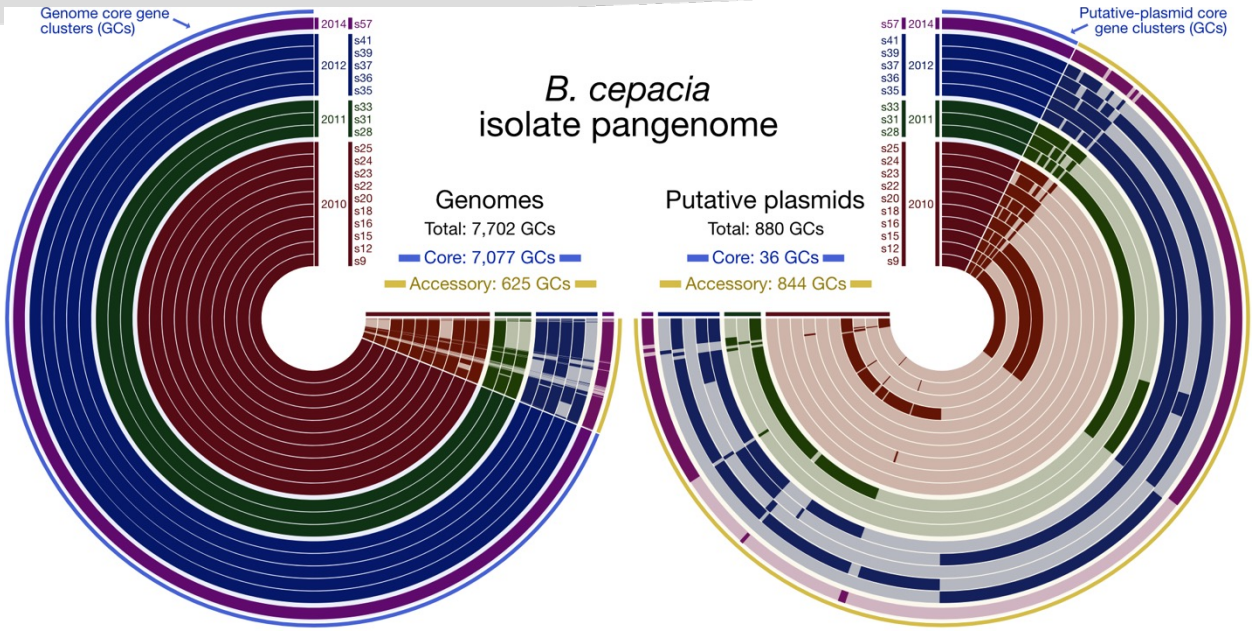
PANGENOMICS : *B. cepacia*

- Analysis of **98 NCBI *B. cepacia*** assemblies and **19 ISS PWD *B. cepacia*** assemblies.
  - ISS isolates are genetically distinct from terrestrial isolates.
  - Most like a previously isolated food pathogen strain.

- The ISS isolates are set apart form terrestrial strains of *B. cepacia* by **distinct flexible genomic islands**
  - **These islands contain elements commonly found on conjugative plasmids.**



B. cepacia plasmids harbor virulence factors

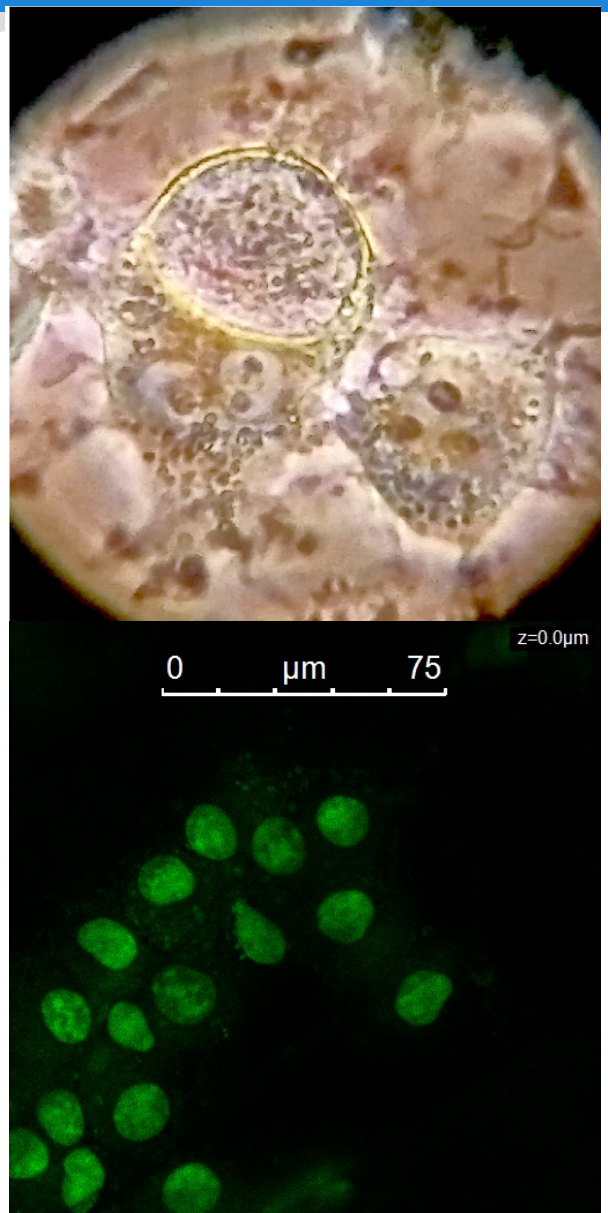


COG functional annotation	Mean copy # ± 1SD
Arsenite efflux pump ArsB, ACR3 family	1.89 ± 1.10
Catechol 2,3-dioxygenase	1.74 ± 0.87
DNA replication protein DnaC	3.00 ± 0.00
Integrase	2.47 ± 0.51
Lysophospholipase, alpha-beta hydrolase superfamily	1.00 ± 0.00
N-acetylglutamate synthase or related acetyltransferase	1.63 ± 0.83
NAD(P)H-dependent FMN reductase	1.68 ± 0.89
Plasmid stabilization system protein ParE	1.00 ± 0.00
Predicted arabinose efflux permease, MFS family	2.42 ± 1.17
Protein involved in initiation of plasmid replication	1.11 ± 0.32
Site-specific DNA-cytosine methylase	1.00 ± 0.00
Transposase	12.42 ± 5.52
Type IV secr., VirD4 comp., TraG/TraD family ATPase	1.11 ± 0.32

Lysophospholipase generates cytotoxic lysophospholipids

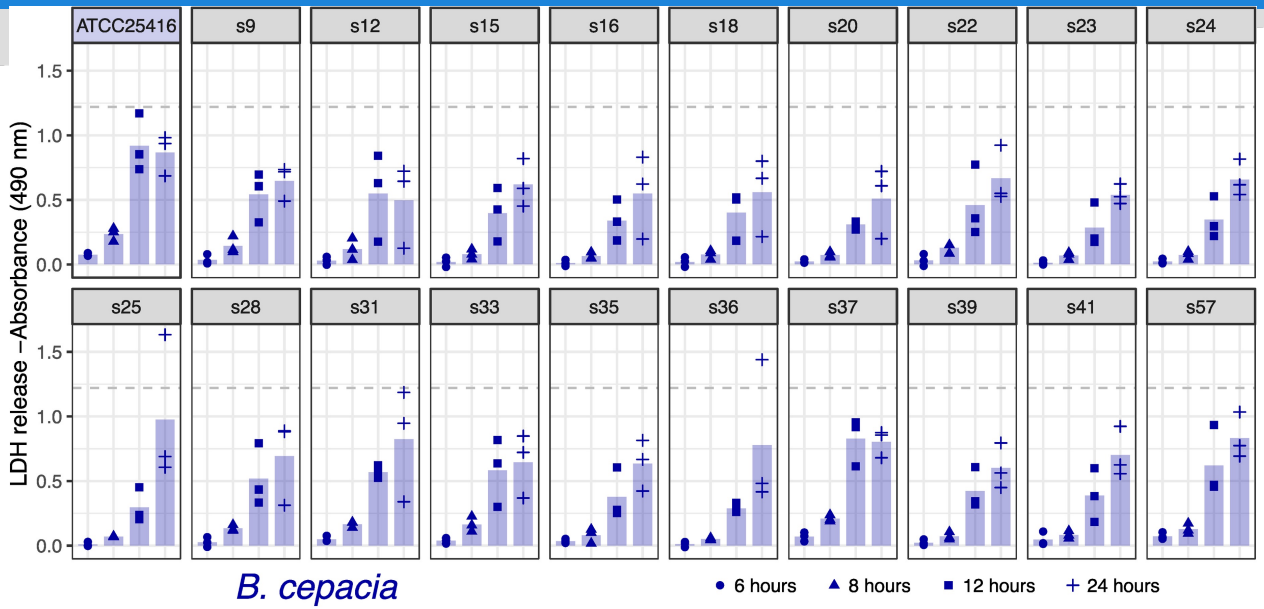
Macrophage infection experiment

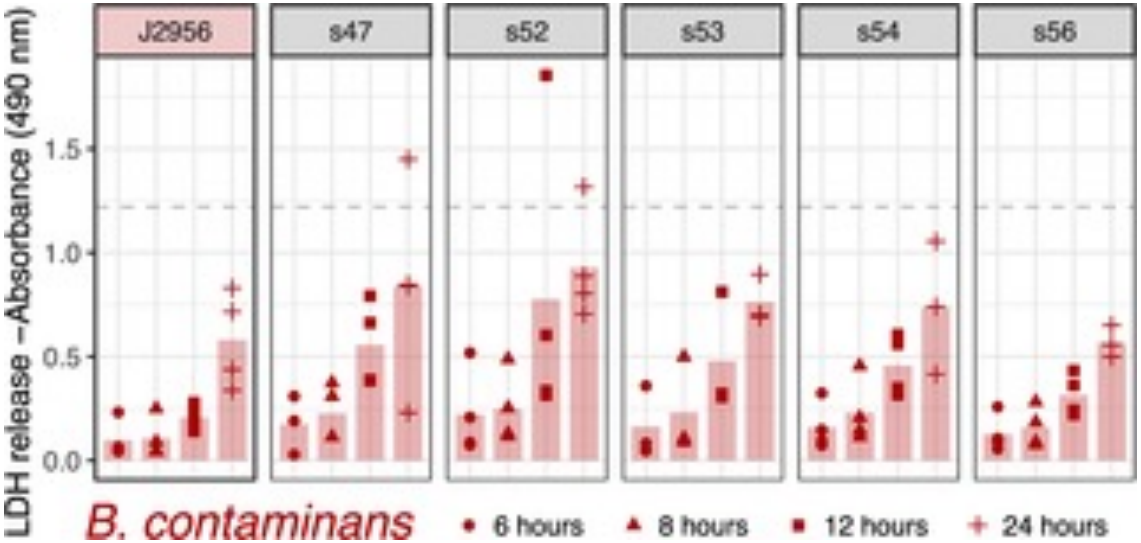
- **Murine macrophage cell line J774A.1** was infected with ISS *Burkholderia* isolates at an MOI of 5 -10 (average of 7).
  - Experiments were conducted in triplicate on three separate days
- 2 hours post infection, cultures were treated with antibiotics
  - 15ug/ml ceftazidime and 1mg/ml amikacin to kill extracellular bacteria



Macrophage cell lysis

by ISS *Burkholderia* isolates



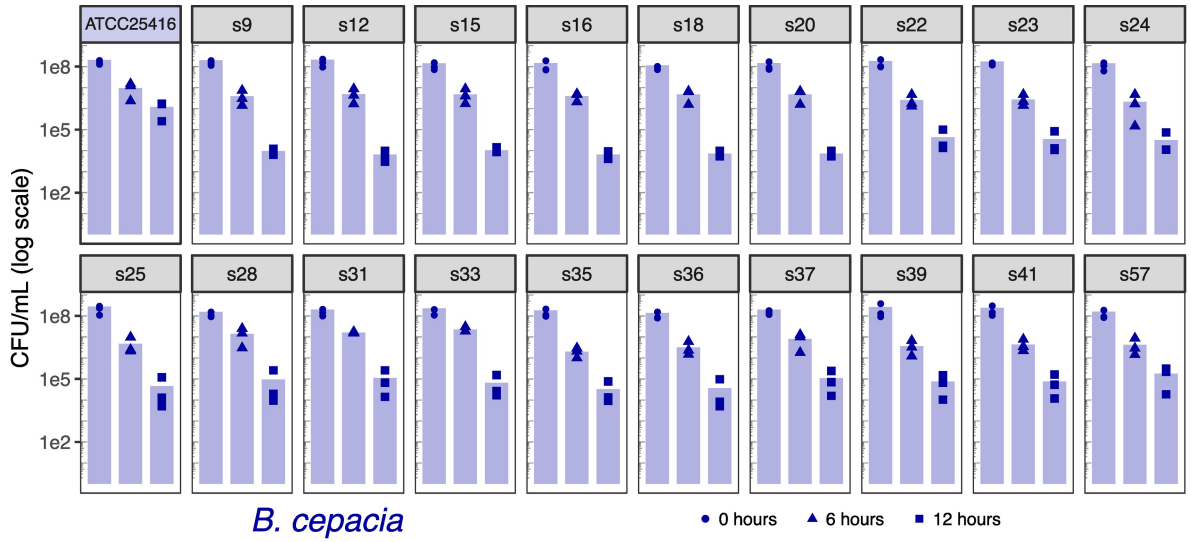


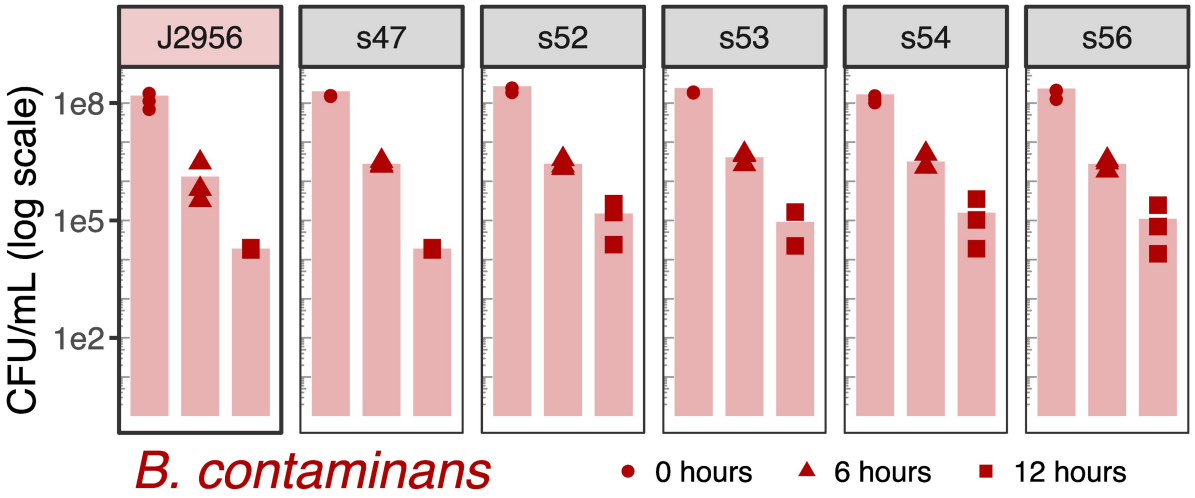
At 6, 8, 12 and 24 hours post-infection, **lactase dehydrogenase (LDH) assay** was performed on supernatants (LDH is released upon macrophage cell lysis)

No isolates are statistically different from each other or controls in the ability to lyse macrophage.

Macrophage infection

invasion and intracellular replication by ISS *Burkholderia*





At 6 and 12 hours post-infection, **intracellularized bacteria** were freed from macrophage and **plated for CFU/ml counts.**

No statistical difference between ISS isolates and *B. cepacia* ATCC and *B. contaminans* control intracellular replication.

ISS isolates remain susceptible to antibiotics used in clinical BCC treatment

Ceftazidime-
bactericidal
3rd generation β-lactam
Cell wall synthesis inhibitor
Broad-spectrum

Cefotaxime-
bactericidal
3rd generation β-lactam
Cell wall synthesis inhibitor
Broad-spectrum

Chloramphenicol-
bacteriostatic
Proteins synthesis inhibitor
(50S)
Broad-spectrum

ug/mL	ceftazidime	cefotaxime	chloramphenicol	ciprofloxacin	cotrimethoprim	levofloxacin	minocycline	meropenem
B. cepacia ATCC25416	8--16	8--32	4--8	0.25--2	16--32	0.25--2	1--4	8--32
s9	8	16--32	2--12.5	1--2	1--32	4--8	4--16	32
s12	4	4--64	4--12.5	0.5--1	8--16	0.5--8	2--4	16--32
s16	4	2--64	8--12.5	0.5--1	8--32	0.5--8	2--8	4--16
s18	4	4--64	8--12.5	0.5--1	8--32	0.5--8	2--16	4--32
s20	8	4--32	8--25	1	16--32	0.5--8	2--16	4--32
s22	16	16	4--25	0.5--1	16--32	4--16	4--8	32
s23	4	8--32	1--25	1--2	16--32	4--16	4--16	16
s24	4	8--64	4--25	1--2	1--32	4--32	4--32	8--32
s25	8	8--16	4--12.5	1	16--32	4--8	4--16	16--32
s28	4	4--32	4--12.5	1--2	16--32	4--32	4--32	8--32
s31	8	16--32	4--12.5	0.5--1	16--32	4--16	4	16--32
s33	4	2	4	0.25	1--2	0.125--0.25	1--2	4--16
s35	8	64	4--8	1	32--64	1--4	8	32
s36	4	64	4--32	1--2	16--32	0.5--2	4--16	32--64
s37	8	64	2--8	1--2	32	1--2	4--8	32
s39	8	32	2--8	1--2	16--32	1--2	4	32--64
s40	4	2--4	16--32	0.125--0.25	4	1--2	4--16	64
s41	8	64	2--8	1	16--32	0.5--1	4--16	32--64
s57	8	8	8	1	16	0.5--1	2	4--8
B. contaminans DSMZ								
s47	8	4--8	4--8	1	8	0.5	2	4
s52	8	16	8	1	4--8	0.5	2	4
s53	8--16	16--32	8	0.5--1	8	0.5	2	4
s54	8--16	8	8	1	4--8	0.25--0.5	2	4
s56	8	16	8	0.5--1	8--16	0.5	2--4	4--8

Meropenem-
bactericidal β-lactam
Cell wall synthesis inhibitor
Broad-spectrum

Minocycline-
bactericidal tetracycline
Protein synthesis inhibitor
(30S)
Broad-spectrum

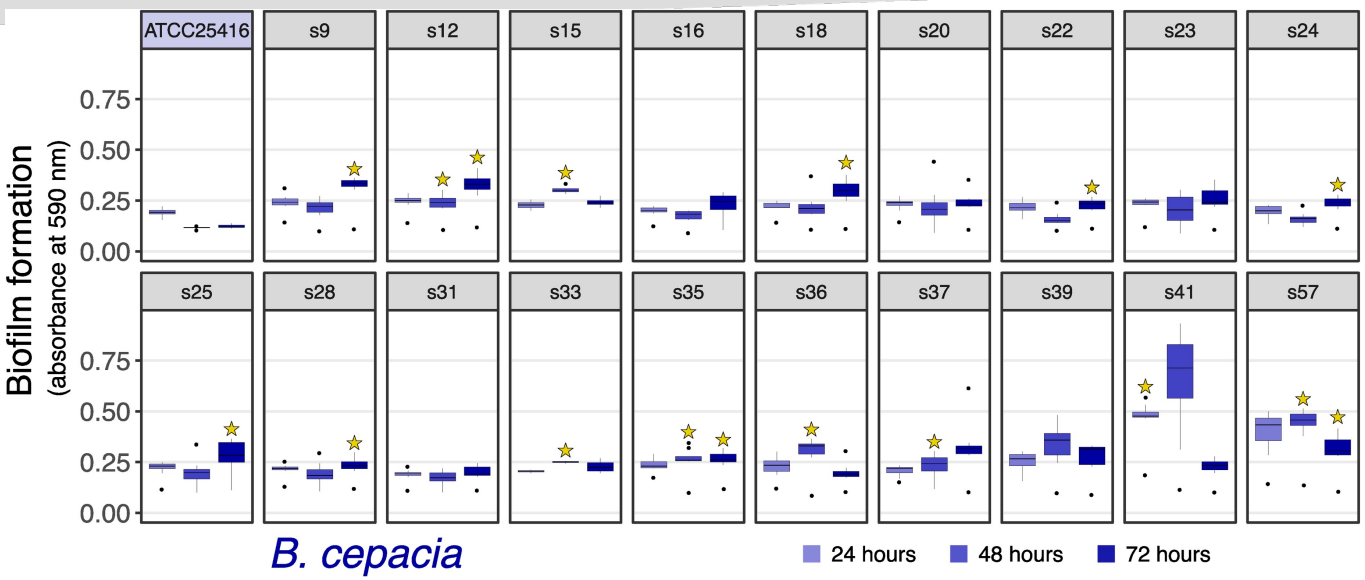
Levofloxacin-
3rd generation flouroquinolone
Cell wall synthesis inhibitor
(DNA gyrase)
Broad-spectrum

Ciprofloxacin-
Fluoroquinolone
DNA synthesis inhibitor
(gyrase and topoisomerase)
Broad-spectrum

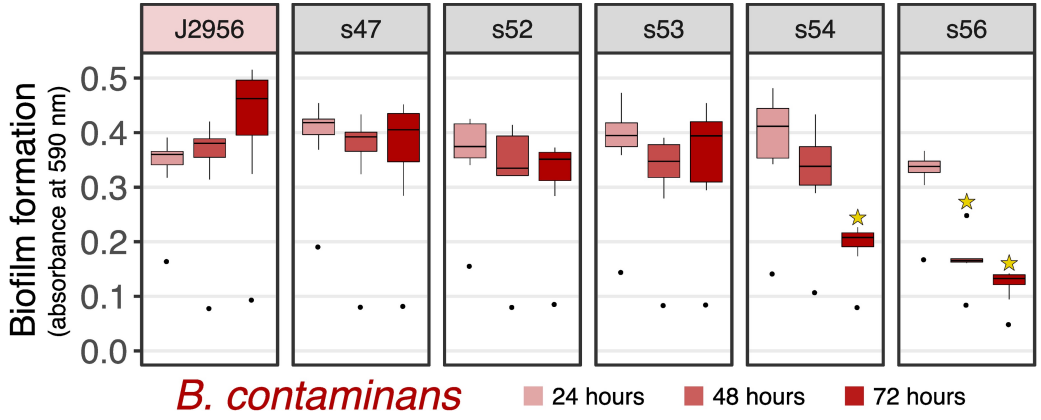
Cotrimethoprim
Trimethoprim/Sulfamethoxozole
(1:5) –
bacteriostatic
binds dihydropteroate in folate
synthetase
DNA synthesis inhibitors

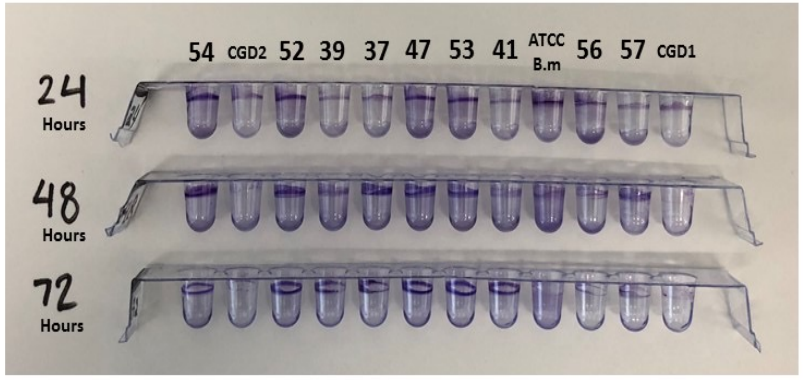
Trimethoprim-
Bacteriostatic
binds to dihydrofolate reductase
DNA synthesis inhibitor

Biofilm formation likely lends to survival in the water system

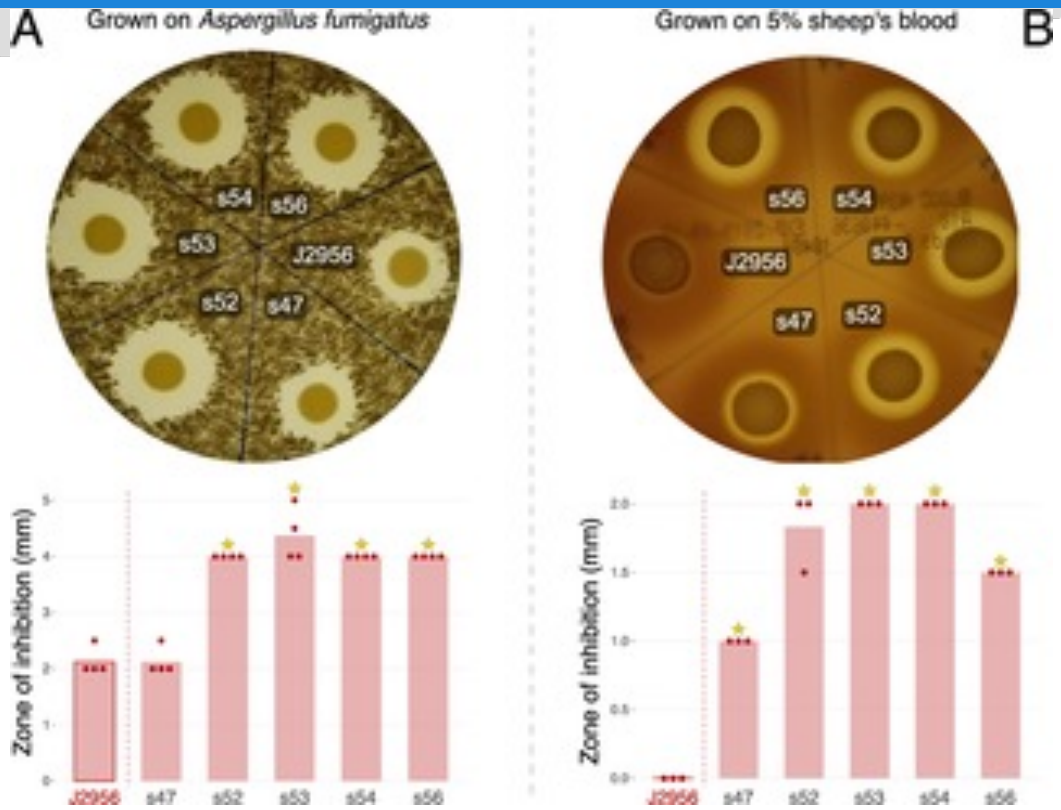


Crystal violet staining for biofilm production in select ISS *Burkholderia* isolates relative to the terrestrial control ATCC strain.





ISS *B. contaminans* demonstrate antifungal and hemolytic properties



Fungal inhibition

ISS *B. contaminans*- known to produce the antifungal occidiofungin- zones of inhibition of formed on *Aspergillus fumigatus*.

Hemolysis assay

zones of inhibition presented by ISS *Burkholderia contaminans* on TSB plates with 5% sheep's blood.

ISS BURKHOLDERIA SUMMARY

- ISS *B. cepacia* isolates **harbor conjugative plasmids** which contain **potential virulence factors**
- ISS *B. cepacia* **isolates invade and replicate within macrophage.**
- Isolates **remain susceptible to all antibiotics** used to clinically treat BCC infections.
- ISS *Burkholderia* likely persist in the water system by their ability to **readily form biofilms**.

Conclusion

- Whole genome sequencing (WGS) level characterization is key to the functional understanding of ISS bacteria.
- Astronauts are microbial vectors.
- ISS isolates of *S. epidermidis* and *Burkholderia* display virulence, but less than or no more than that of the reference terrestrial controls.

Acknowledgements

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Kristine Khieu, Victoria Thai, Henry Lee, Sydney Anderson, Shirli Cohen

2019 UCSD Senior Design Team:

Grace Lee, Kysha Mercader, Marissa Vecina

Publications

> iScience. 2021 Jan 29;24(2):102114. doi: 10.1016/j.isci.2021.102114. eCollection 2021 Feb 19.

Reference-guided metagenomics reveals genome-level evidence of potential microbial transmission from the ISS environment to an astronaut's microbiome

Michael D Lee [1 2], Aubrie O'Rourke [3], Hernan Lorenzi [4], Brad M Bebout [1], Chris L Dupont [5], R Craig Everroad [1]

Affiliations + expand

PMID: 33659879 PMCID: PMC7892915 DOI: 10.1016/j.isci.2021.102114

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RESEARCH ARTICLE

Genomic and phenotypic characterization of *Burkholderia* isolates from the potable water system of the International Space Station

Aubrie O'Rourke, Michael D. Lee, William C. Nierman, R. Craig Everroad, Chris L. Dupont

Published: February 19, 2020 • https://doi.org/10.1371/journal.pone.0227152