Bacterial Monitoring in the International Space Station—“Kibo”

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Space habitation: life in a closed environment under microgravity

- Pathogenicity of some bacteria increases under microgravity (Wilson, 2007)
- Immune dysfunction occurred associated with space flight (Borchers, 2002)
- Microbes caused troubles in systems and materials in Space Station Mir

=> Relationship between humans and microbes may change in space habitation

“How and where do microbes proliferate in ISS?”

Analysis of microbial dynamics in ISS is important for

i) Operating ISS with microbiological safety

ii) Providing essential information for long stay in space

   e.g.) define correct upper and lower thresholds of air, surface, water, …

and

New techniques available in ISS can be valuable on ground.
New methods for microbial monitoring in ISS

1) Sampling with swab: used by NASA and other space agencies  
   => rather troublesome --- open cap, take wet wipe, wiping, …
2) Culture-independent methods should be used, because more than 90% of bacteria in environments cannot be cultured under conventional conditions.
Adhesive sheet for microbial monitoring in “Kibo”

Cover sheet (polyethylene terephthalate)

Clean face

Adhesive area 25 mm × 25 mm

Adhesive [sterilized by gamma radiation]

New culture-independent methods for determination of bacterial abundance and phylogenetic affiliation

Direct DNA extraction
  - Swab sample: freeze-thawing and enzymatic extraction
  - Adhesive sheet: beads beating (mechanical cell disruption)

Determination of bacterial abundance
  - Fluorescence microscopy (nucleic acid staining with SYBR Green II)
  - Quantitative PCR (targeting 16S rRNA gene)

Bacterial community analysis
  - Nested PCR-DGGE based on 16S rRNA gene sequences
Sampling points in “Kibo”

- Air diffuser
- Handrail
- SAIBO Rack (incubator)
- PC palm rest
Sampling points in “Kibo”

Inside of incubator

Air intake
## Sampling points, date and method in “Microbe”

<table>
<thead>
<tr>
<th>Sampling Points</th>
<th>Microbe-I</th>
<th>Microbe-II</th>
<th>Microbe-II’</th>
<th>Microbe-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling Method</td>
<td>Swab</td>
<td>Swab</td>
<td>Swab</td>
<td>Swab</td>
</tr>
<tr>
<td></td>
<td>Adhesive sheet</td>
<td>Adhesive sheet</td>
<td>Adhesive sheet</td>
<td>Adhesive sheet</td>
</tr>
<tr>
<td>Incubator</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Air diffuser</td>
<td>○</td>
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<td>○</td>
</tr>
<tr>
<td>Handrail</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Inside of incubator</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
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<tr>
<td>Air intake</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Palm rest</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
## Bacterial abundance in interior surface in “Kibo”: Microbe-II (29 Oct. 2010)

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Total bacteria (cells/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluorescence Microscopy $^a$</td>
</tr>
<tr>
<td>Incubator</td>
<td>- ($&lt; 2 \times 10^4$)</td>
</tr>
<tr>
<td>Handrail</td>
<td>± ($3 \times 10^4$)</td>
</tr>
<tr>
<td>Air intake</td>
<td>Not countable</td>
</tr>
<tr>
<td>PC palm rest</td>
<td>± ($3 \times 10^4$)</td>
</tr>
</tbody>
</table>

+ : positive  
± : same order of magnitude as quantification limit  
- : below quantification limit

Quantification limit: Fluorescent microscopy : $2 \times 10^4$ cells/cm²

Sampling: adhesive sheet  
$^a$ Dye: SYBR Green II (nucleic acid stain)  
$^b$ Target : 16S rRNA gene (calculated as 4 copies/cell)  
Primers: EUB f933, EUB r1387

**Phylogenetic affiliation of bacteria collected in “Kibo”: Microbe-II (29 Oct. 2010)**

Sampling: adhesive sheet

Phylogenetic affiliation was determined with sequencing following 16S rRNA gene-targeted nested PCR-DGGE.

Study plan of Microbe-IV

- Four times sampling during FY2014 to FY2016

- Sampling point:
  (as Microbe I, II and III) handrail, surface and inside of incubator, air intake, air diffuser, laptop PC palm rest
  (New point) handrail on entrance, wall of “Kibo”, foot hold, MELFI (freezer) door

- Sampling: swabbing and adhesive sheet

- Analysis

  <Bacteria>
  Abundance --- Quantitative PCR and fluorescence microscopy
  Community --- Pyrosequencing

  <Fungi>
  Abundance --- Quantitative PCR
  Community --- Pyrosequencing
Microbial monitoring for long stay in space habitat

Rapid monitoring:

i) total bacterial number

ii) Number of harmful bacteria

Bring samples to laboratory for analysis …

↓

“Real-time and on-site” microbial monitoring is required
On-chip staining of bacterial cells with microfluidic device

Sample: *Legionella pneumophila*
Staining: anti-*L. pneumophila* fluorescent antibody
New portable microfluidic system for on-site bacterial monitoring

New portable system
(36 cm × 54 cm × 23 cm, 15 kg)