



Medizinische Universität Graz

Mykobiom der Lunge

Robert Krause

Section of Infectious Diseases and Tropical Medicine

Department of Internal Medicine

Medical University of Graz

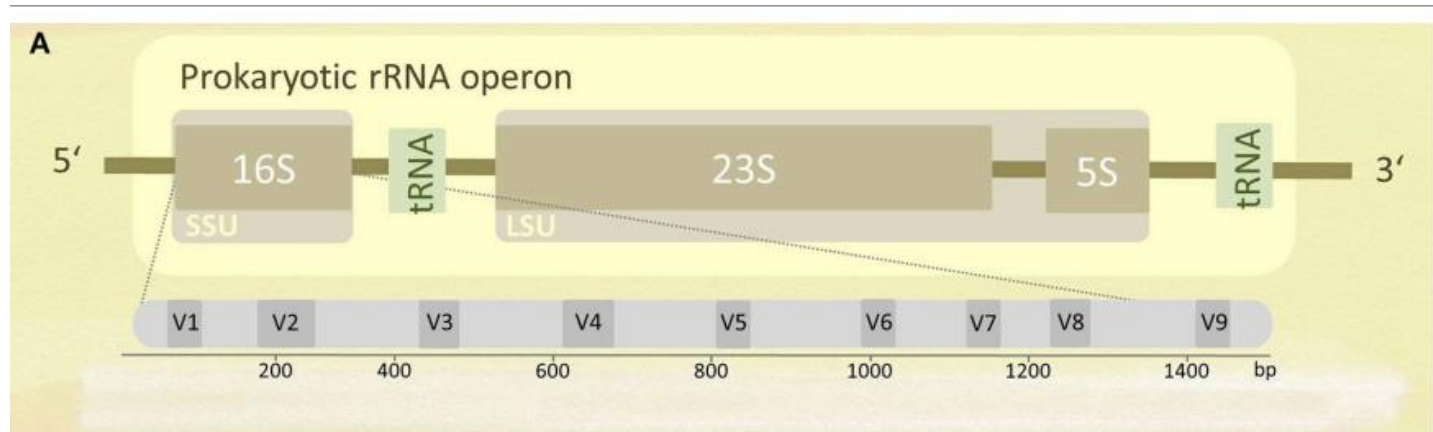
Mykobiom der Lunge

- Können Mykobiomstudien/-untersuchungen...?
 - die Pathophysiologie erklären?
 - Lungenfunktion (Oxygenierung)
 - Obstruktion, Exacerbation der Obstruktion
 - Abstoßung bei LuTX, BOOP...
 - Infektion (Pneumonie...)
 - Diagnosen liefern?
 - Hilfreich bei therapeutischen Entscheidungen und Therapien sein?

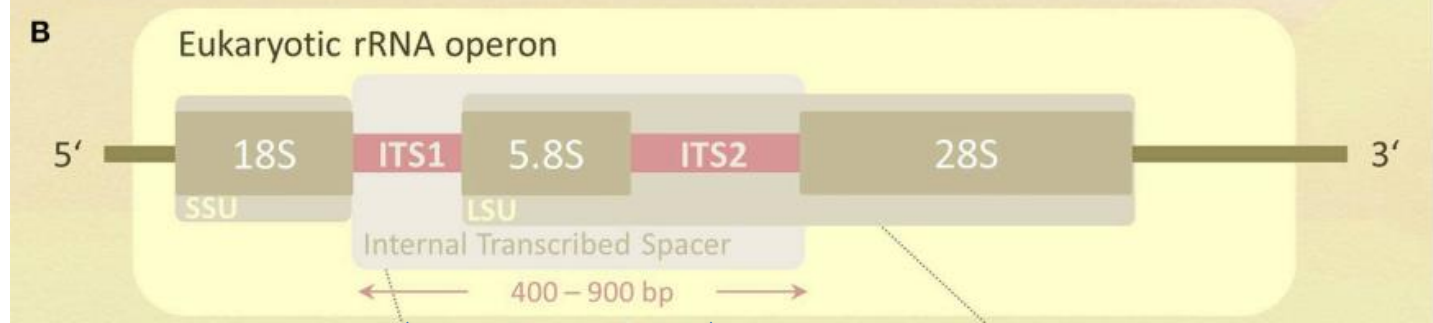
Fungal rDNA regions targeted for mycobiome characterization → ITS

internal transcribed spacer

Bacteria



Fungi



ITS 1

ITS 2

Sampling the lower respiratory tract

- Sputum? (Exhaled breath condensate?*)
 - Sputum passes the oral cavity
 - Mycobiota present in the oral cavity?
 - Possible contamination of LRT samples

Oral Mycobiome in healthy adults

- 20 healthy adults
 - Non-smoking
 - No recent antifungal therapy (not further defined)
 - No oral mucosal disease, no steroids, no pregnancy, no IDDM
-
- 15ml oral wash with saline
 - ITS1 PCR and 454 pyrosequencing

Oral Mycobiome in healthy adults

Fungal genera

Oral Mycobiome of Healthy Individuals

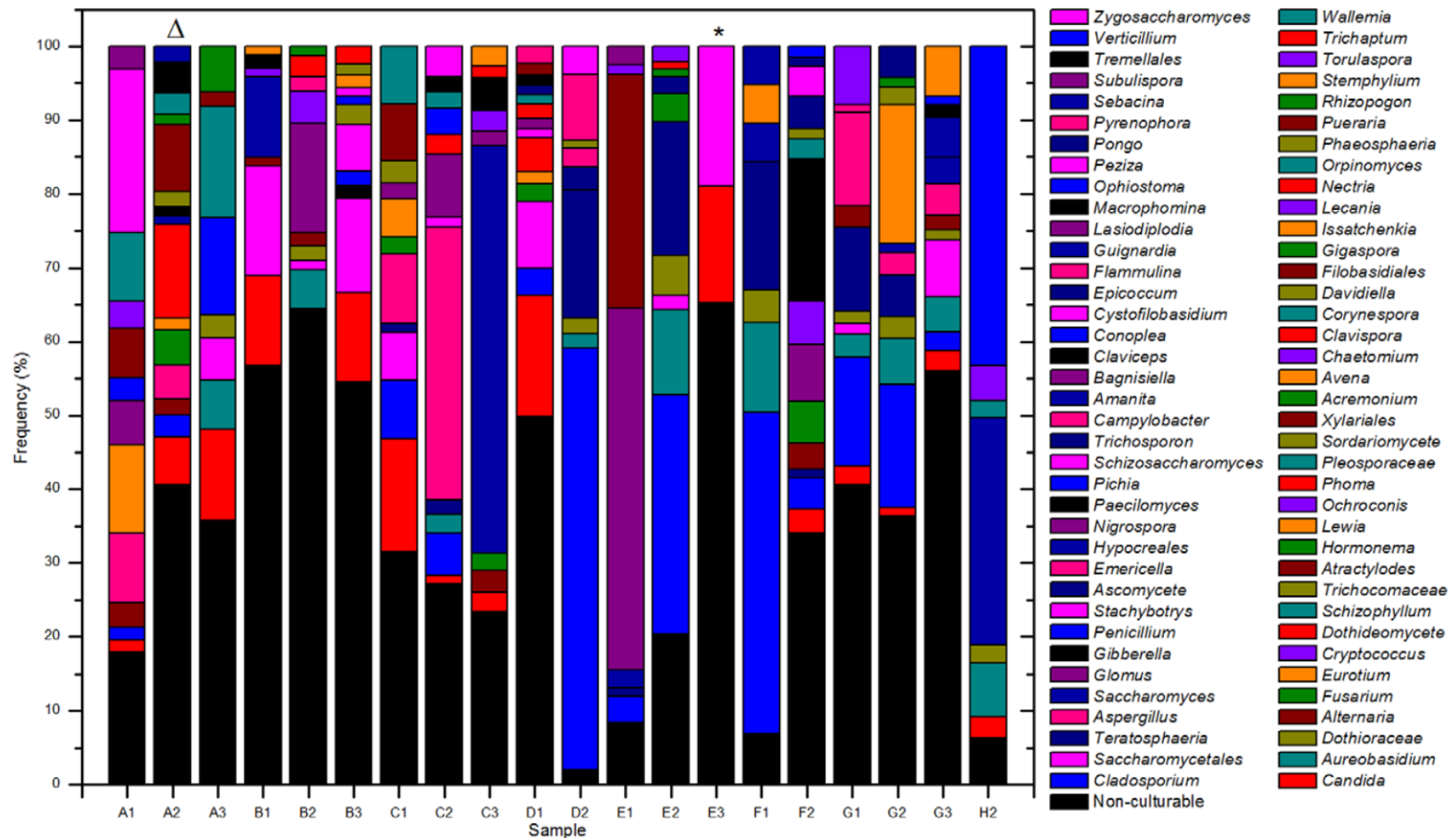


Figure 2. Overall distribution of fungi in oral rinse samples obtained from 20 healthy individuals. The triangle and asterisk indicate samples containing 16 and 3 fungal genera, respectively. See Table 1 for sample details.
doi:10.1371/journal.ppat.1000713.g002

Oral Mycobiome in healthy adults

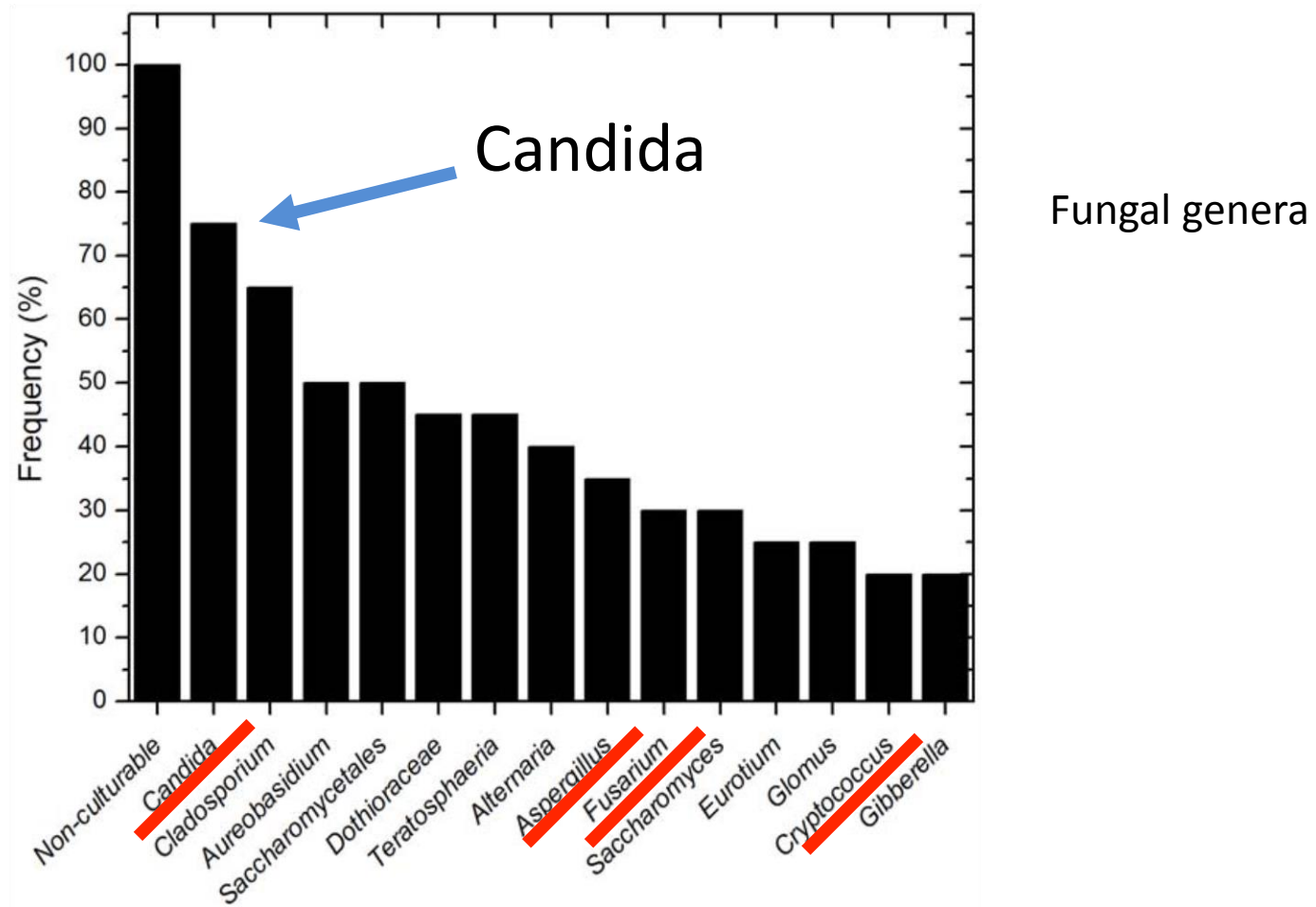


Figure 3. Frequency of fungal genera present in more than 20 percent of the tested samples.
doi:10.1371/journal.ppat.1000713.g003

Sampling the lower respiratory tract

- Sputum?
- Sampling of lower respiratory tract **mucus**
 - Bronchoscopic guided lavage (contains lavage fluid and epithelial lining fluid)
 - Blind suction of lower respiratory tract mucus (Tracheobronchial aspirate)
- Sampling of lower respiratory tract/lung **tissue**
 - Alive patients
 - Bronchoscopic guided biopsy
 - Transthoracic puncture and biopsy
 - Surgical resection
 - Autopsy in deceased patients (post mortem examination)

Sampling the lower respiratory tract

- Sampling of lower respiratory tract mucus by tubes or bronchoscope
 - → oral cavity and upper respiratory tract has to be passed
 - Contamination of sampling material
- Sampling of Lung Tissue

Sampling the lower respiratory tract

- Sampling of Lower respiratory tract mucus
 - → oral cavity and upper respiratory tract has to be passed
- Sampling of lung tissue
 - → serious side effects
 - Alive patients
 - Bronchoscopic guided biopsy → bleeding, pneumothorax
 - Transthoracic puncture and biopsy → bleeding, pneumothorax
 - Surgical resection → too invasive, necessary only in rare cases for investigation of interstitial lung diseases
 - During autopsy (post mortem examination) → too late?
 - Alterations of microbiota/mycobiota after death?
 - No healthy control group possible
 - Too invasive

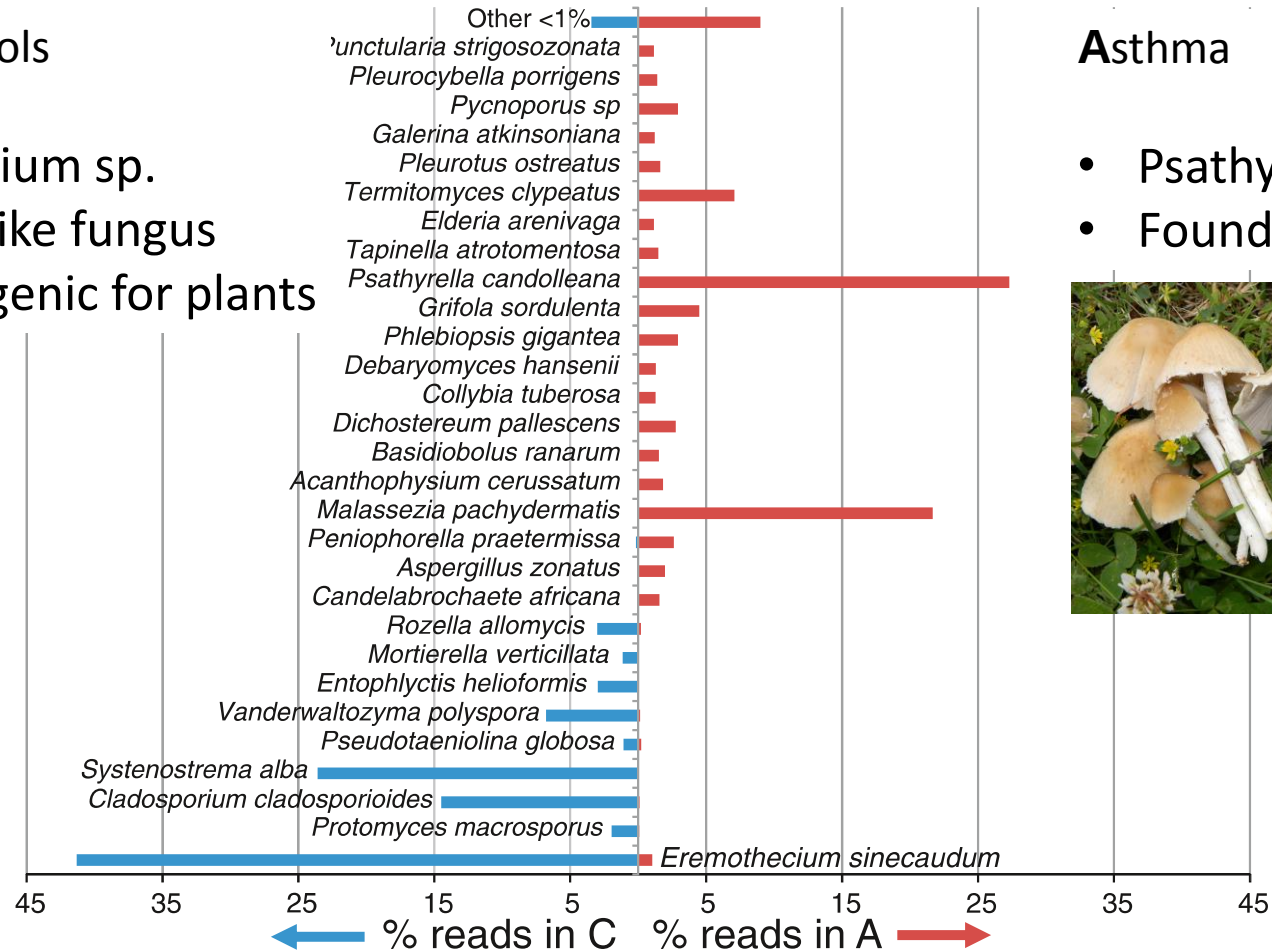
Mycobiome in asthma patients

- **Pooled** sputum samples
- 30 asthma patients and 13 non-atopic control subjects
 - „A sample from one asthma patient was inadvertently included in the control set“
- Patients were free of oral steroid use ≥ 2 weeks prior to induced sputum sampling but most of them were on inhaled corticosteroids
- No information regarding previous or current antibiotic or antifungal therapy
- 18S rRNA PCR, 454 pyrosequencing

Mycobiome in asthma patients

Healthy Controls

- *Eremothecium* sp.
 - Yeast like fungus
 - Pathogenic for plants



Asthma

- *Psathyrella* sp.
- Found on lawns



Figure 1 Graph showing the percentage of pyrosequencing reads for fungal species identified in the Asthma patient (A) or control participant (C) samples (species identified for reads greater than 1%).

...data used for reviews

Attempts for reliable sampling of the lower respiratory tract (LRT)

- Sampling of lower respiratory tract mucus
 - 2 bronchoscopy technique
 - Sequential sampling → not for routine
 - or tube in tube technique → not for routine
 - Sampling only intubated patients
 - Sampling oral cavity **and** LRT and calculate LRT microbiota/mycobiota

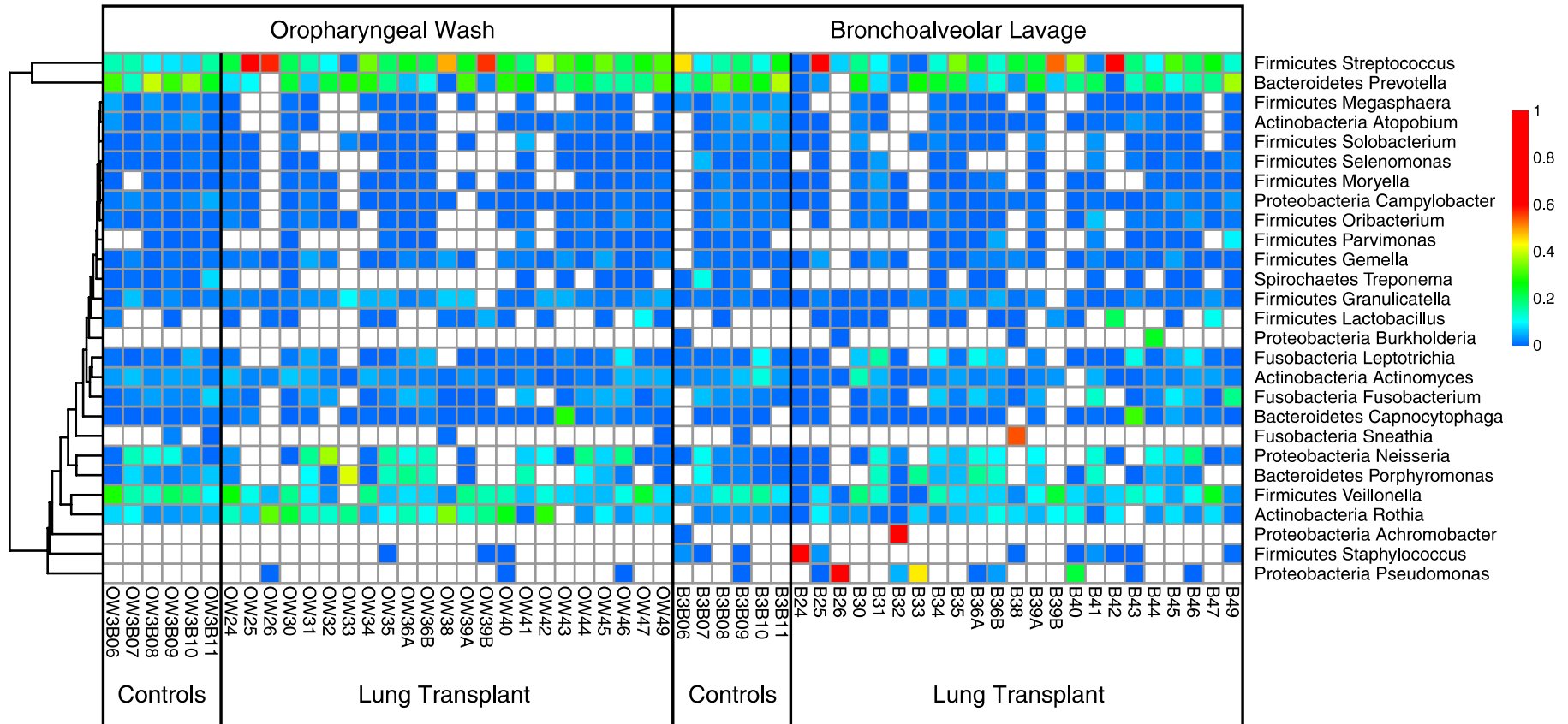
Mycobiome in LRT

- Cystic fibrosis
- Asthma
- Lung Transplant
- **ICU patients**

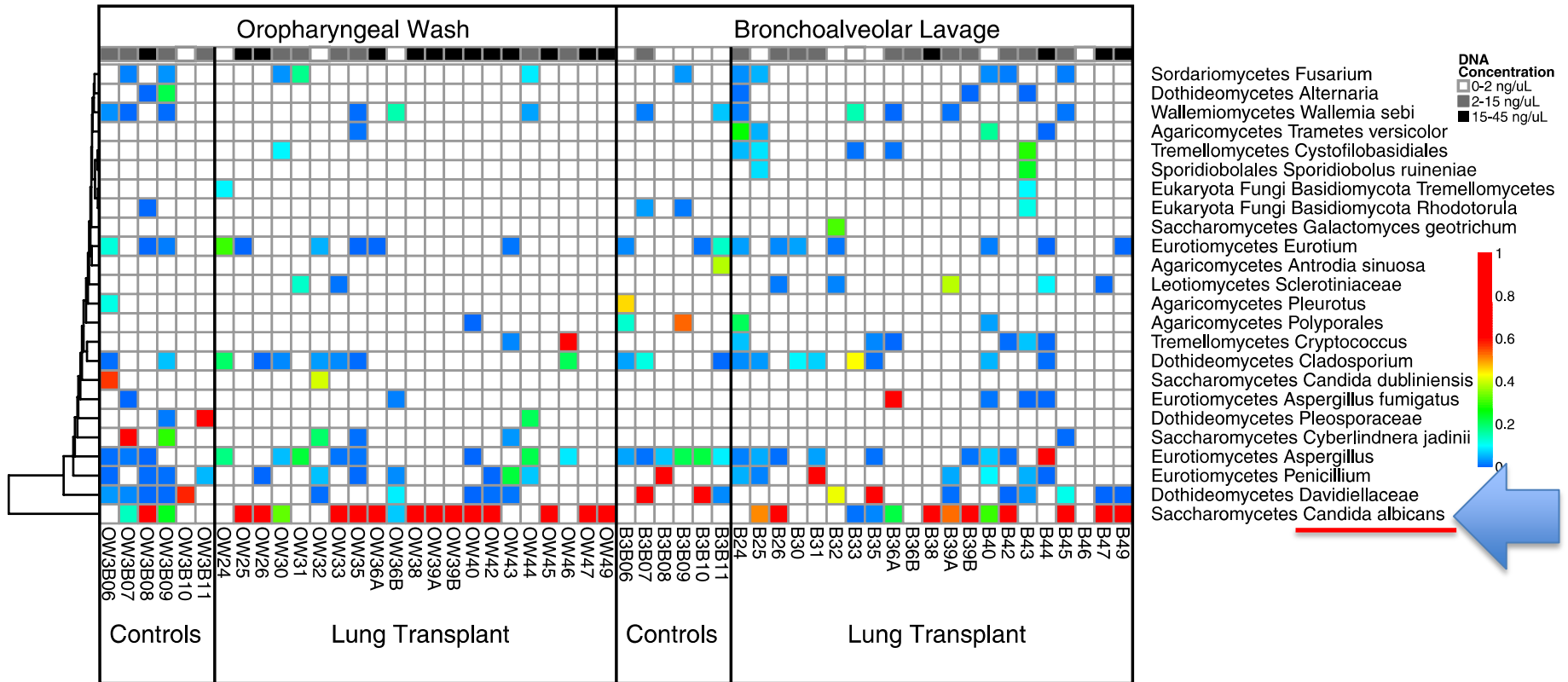
Microbiome and mycobiome in Lung Transplant (TX) Patients

- 21 Lung TX patients
- All had immunosuppressive and antibiotic therapy
- three received voriconazole and six nystatin (one patient had both antifungals)
- Oral wash and bronchoscopic guided BAL
- No functional assessment of lungs
- 16S rDNA and ITS1F/ITS2 PCR and 454-pyrosequencing

Microbiome in Lung Transplant Patients



Mycobiome in Lung Transplant Patients



Bacterial and fungal richness and diversity of airway communities were reduced in transplant subjects compared with control subjects.

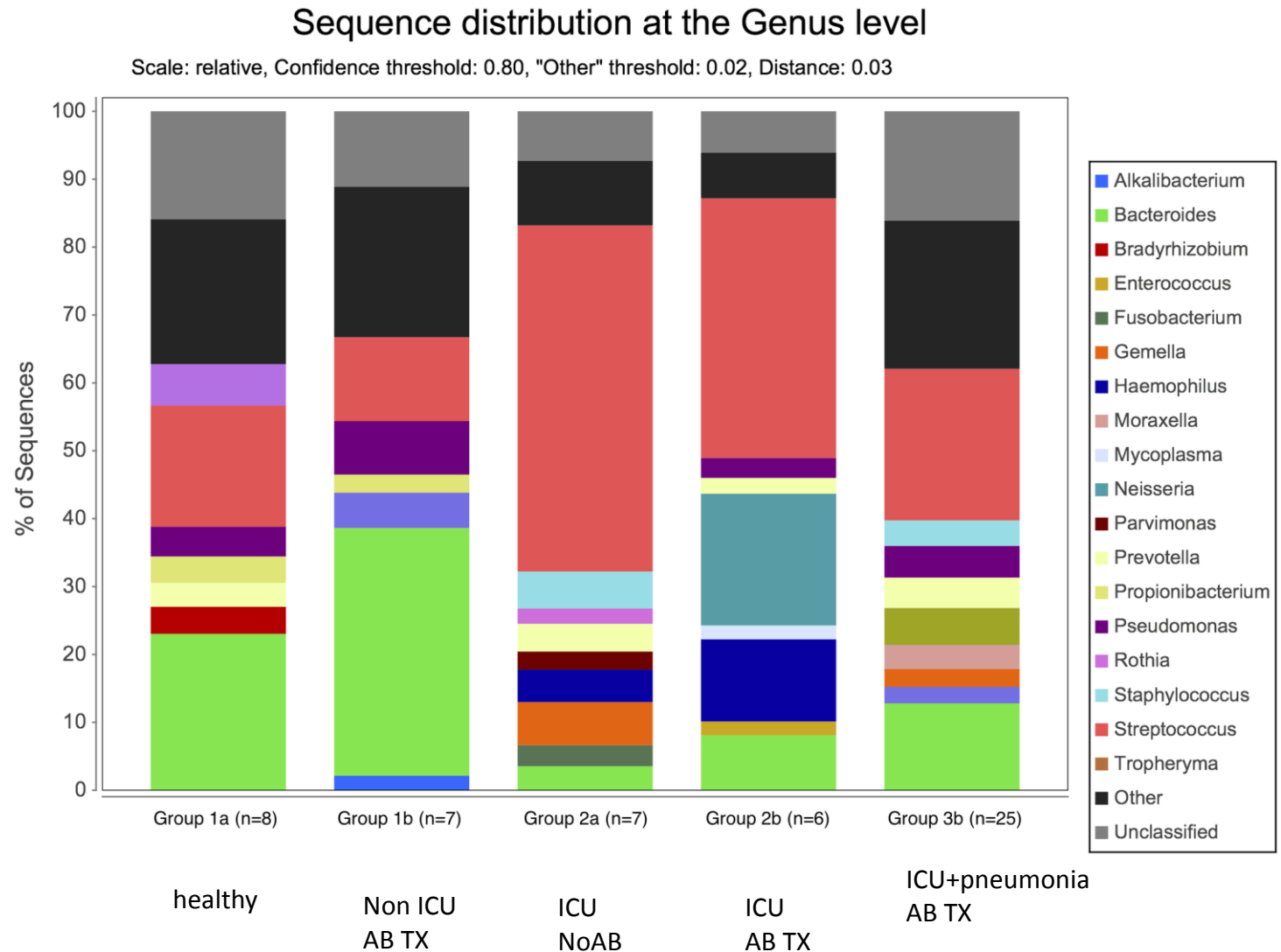
Microbiome and **mycobiome** in Lung Transplant Patients

- Bacterial and fungal richness and diversity of airway communities were reduced in transplant subjects compared with control subjects
- Candida increased
- Consequence?
 - Influence on lung function?
 - Graft failure?
 - Trigger of bronchiolitis obliterans syndrome (BOS)?

Microbiome and **mycobiome** in ICU patients

- 53 subjects, 5 groups
 1. Healthy controls (sampled during anaesthesia for plastic surgery)
 2. Lung healthy but AB therapy
 3. ICU, no AB therapy
 4. ICU, AB therapy but no pneumonia
 5. ICU, AB therapy and pneumonia
- All groups without antifungal therapy
- sampling via orotracheal tubes (incl healthy controls)
 - median day 2/3 after admission to ICU
- 16S rRNA and ITS PCR, 454 sequencing

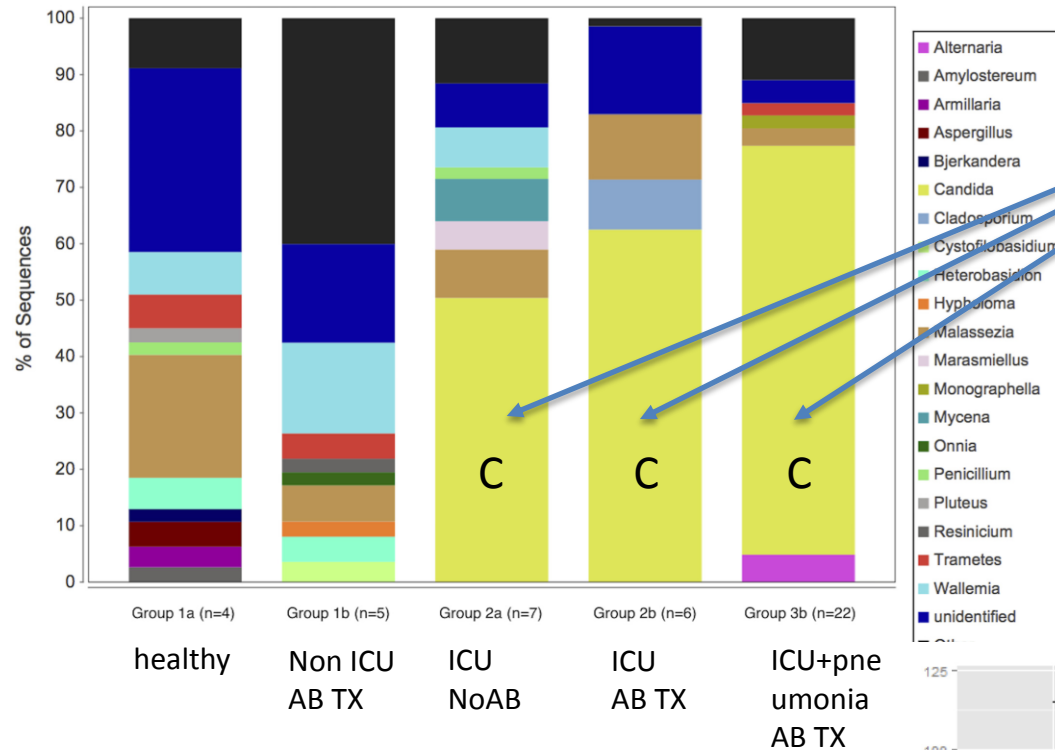
Microbiome in ICU patients



Mycobiome in ICU patients

Sequence distribution at the Genus level

Scale: relative, Confidence threshold: 0.80, "Other" threshold: 0.02



C = Candida

Average relative Candida abundance

