

AMPLICON SEQUENCING REVEALS BACTERIA DIVERSITY OF INDOOR AIR MICROBIOME IN HOSPITAL BUILDINGS

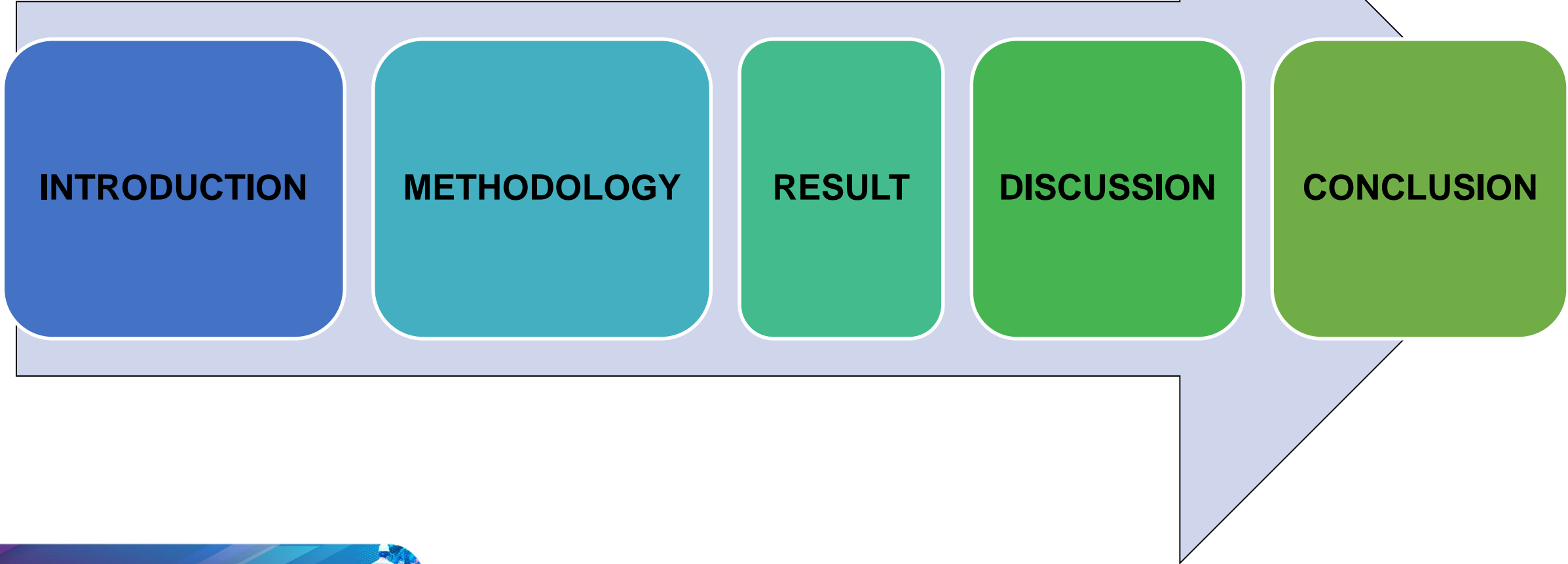
Presenter:

Nor Husna binti Mat Hussin

Faculty of Science and Industrial Technology

Universiti Malaysia Pahang Al- Sultan Abdullah (UMPSA)





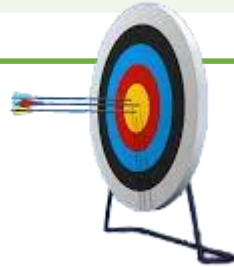


INTRODUCTION

Research Overview



Research Objectives



Problem Statement



Significance of study



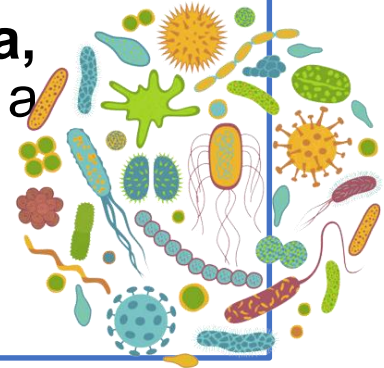
Amplicon Sequencing:

- Techniques in **molecular biology**
- Analyze and sequence-specific DNA fragments of microorganisms. (Gangneux et.al., 2020).
- Applied **high-throughput sequencing – known as (NGS)** and **analyse the microbes DNA, extracted directly from environmental samples**



Indoor air microbiome:

- The collections of the **microbiological community** including **bacteria, fungi, viruses, protozoa and archae** that constitute the indoor air of a building (Yang et. al., 2023)
- Affect human health
- 70- 90% indoor



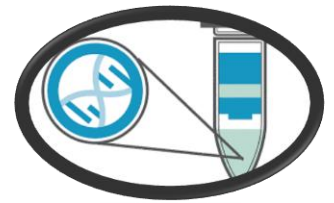


Harbor wide range of air microbiome- acts as a reservoir for pathogenic organisms

Pathogenic, non-pathogenic, opportunistic, normal flora/commensal, and protective microbes

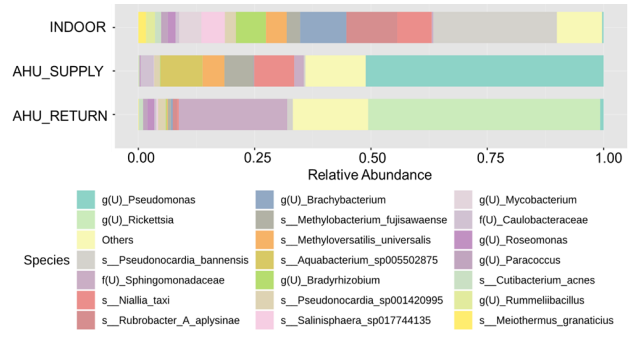
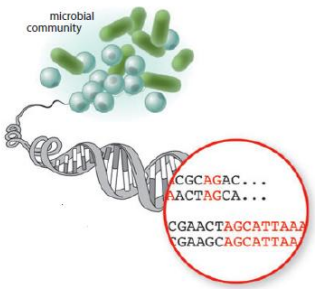
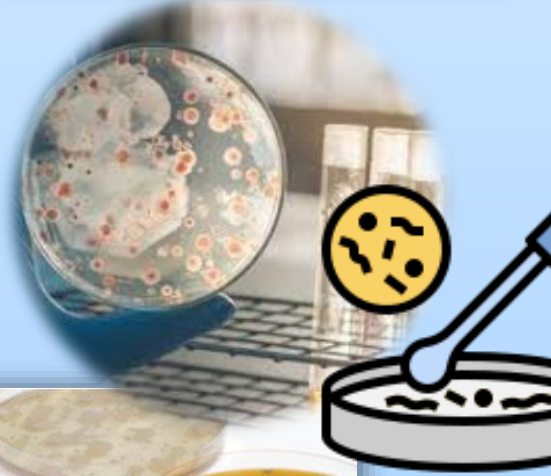
Nosocomial infections or HAI to healthcare workers and patients

Pathogenic and opportunistic pathogens



DNA EXTRACTION

CULTURE-DEPENDENT VS CULTURE-INDEPENDENT METHOD





CULTURE-DEPENDENT METHOD

VS

CULTURE-INDEPENDENT METHOD

Depending on the **culture media and types of media** (to cultivate bacteria in the lab)

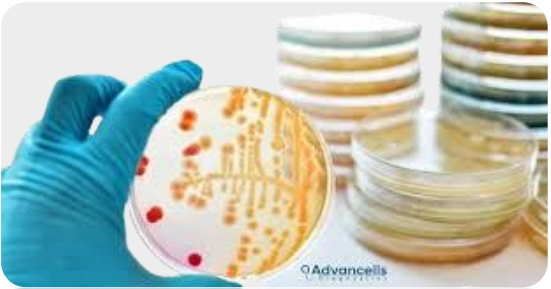
High sensitivity but low specificity- inconclusive findings and biased

Laborious and longer time (incubation period, biochemical test and microscopic examination)

Bacteria genomic DNA- analyze directly from the environmental samples

High sensitivity and specificity

Simple and rapid (can analyze a large volume of samples within a short period of time)





Problem statement

Inconclusive findings and biased (not all bacteria are culturable & some slow growth)



Laborious and long-time (incubation period, series of biochemical test)



Urgency- reducing the prevalence of NI, reduce extra expenditure and improve hospital management



Prone to contamination- during transportation (inappropriate temperature) and analysis

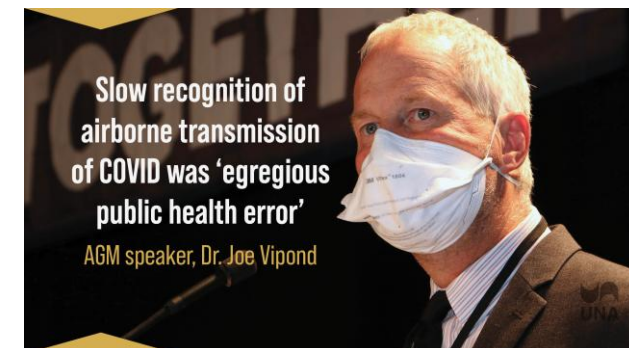


Hospital Teluk Intan ditutup.
37 petugas positif Covid-19
Govt shuts down parts of Teluk Intan Hospital after 37 medical ...



Hospital Teluk Intan Ditutup Akibat 37 Petugas Positif COVID-19?

SEBENARNYA.MY



To optimize **culture-independent method** in the sampling of indoor air microbiome

To profile **bacterial community** in indoor air at the hospital by using the **amplicon sequencing method**.



Application of the new method

- Supplementary method and analysis for IAQ assessor and other personnel for examining biological contaminants
 - Identification vs concentration
 - ↓ contaminations (transportation & analysis)
- Rapid analysis for large volumes of samples and less laborious

Comprehensive profiling of microbial community

- Include both cultivable and non-cultivable microorganisms
- High sensitivity and specificity
- ↓ waste

Application of the

- Supplementary method and analysis for IAQ assessor
- Identification vs concentration
- ↓ contaminations (transportation & analysis)

laborious

INDUSTRY CODE OF PRACTICE ON INDOOR AIR QUALITY 2010

DEPARTMENT OF OCCUPATIONAL SAFETY AND HEALTH
 MINISTRY OF HUMAN RESOURCES, MALAYSIA

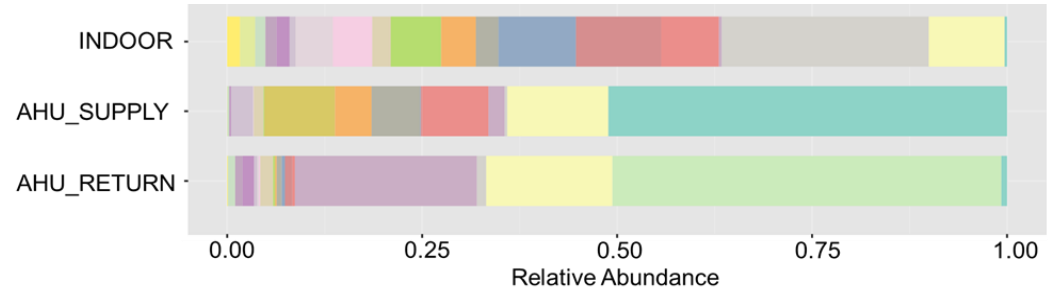
JKKP DP(S) 127/379/4-39

Table 2: List of indoor air contaminants and the acceptable limits

Indoor Air Contaminants	Acceptable limits	
	ppm	mg/m ³
Chemical contaminants		
(a) Carbon monoxide	10	-
(b) Formaldehyde	0.1	-
(c) Ozone	0.05	-
(d) Respirable particulates	-	0.1
(e) Total volatile organic compounds (TVOC)	3	-
Biological contaminants		
(a) Total bacterial counts	-	500*
(b) Total fungal counts	-	1000*
Ventilation performance indicator		
(a) Carbon dioxide	C1000	-

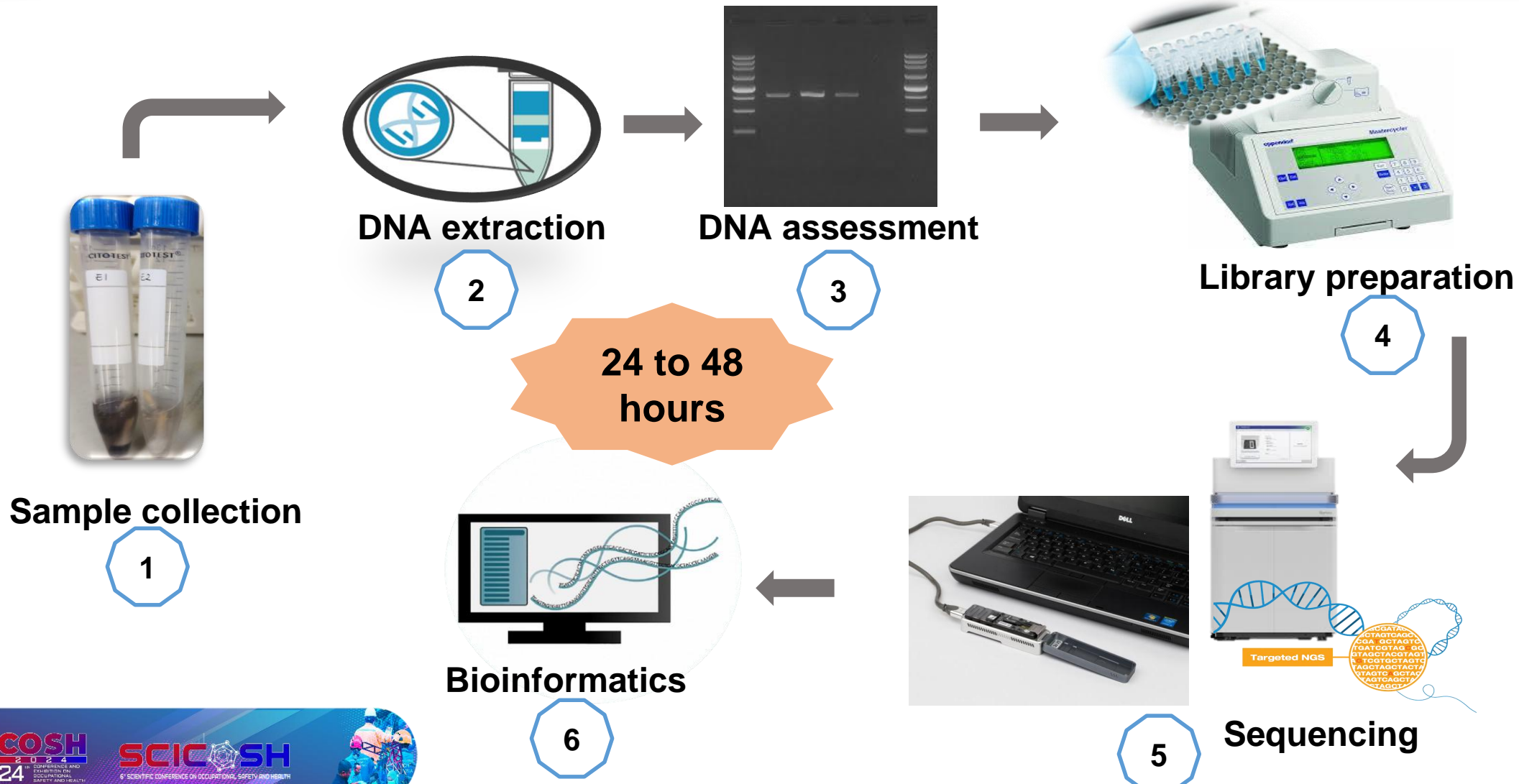
- Notes:
- For chemical contaminants, the limits are eight-hour time-weighted average airborne concentrations.
 - mg/m³ is milligrams per cubic meter of air at 25° Celsius and one atmosphere pressure.
 - ppm is parts of vapour or gas per million parts of contaminated air by volume.
 - cfu/m³ is colony forming units per cubic meter.
 - C is the ceiling limit that shall not be exceeded at any time. Readings above 1000ppm are indication of inadequate ventilation.
 - *excess of bacterial counts does not necessarily imply health risk but serve as an indicator for further investigation.

Contaminant	Indoor	AHU_SUPPLY	AHU_RETURN
Biological contaminants			
(a) Total bacterial counts	-	-	500*
(b) Total fungal counts	-	-	1000*



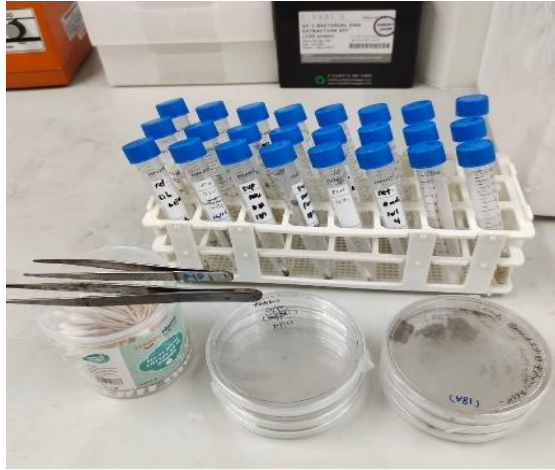
- Species
- g(U)_Pseudomonas
 - g(U)_Rickettsia
 - Others
 - s__Pseudonocardia_bannensis
 - f(U)_Sphingomonadaceae
 - s__Niallia_taxi
 - s__Rubrobacter_A_aplysinae
 - g(U)_Brachybacterium
 - s__Methylobacterium_fujisawaense
 - s__Methyloversatilis_universalis
 - s__Aquabacterium_sp005502875
 - g(U)_Bradyrhizobium
 - s__Pseudonocardia_sp001420995
 - s__Salinisphaera_sp017744135
 - g(U)_Mycobacterium
 - f(U)_Caulobacteraceae
 - g(U)_Roseomonas
 - g(U)_Paracoccus
 - s__Cutibacterium_acnes
 - g(U)_Rummeliibacillus
 - s__Meiothermus_granaticus

METHODOLOGY





METHODOLOGY

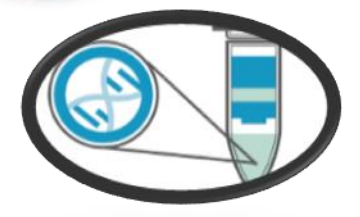


Sample collection

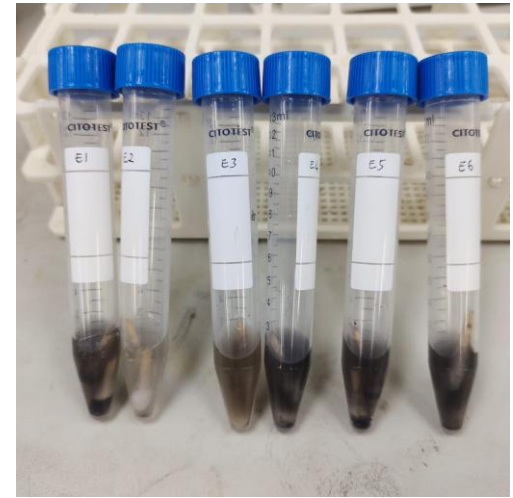


- 1
- 2
- 3
- 4
- 5
- 6





DNA EXTRACTION

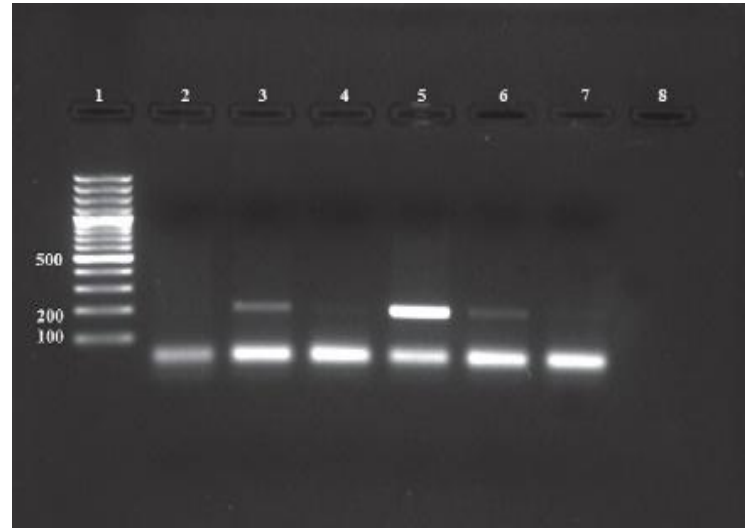


**Bacteria
DNA
extraction**



- 1
- 2
- 3
- 4
- 5
- 6



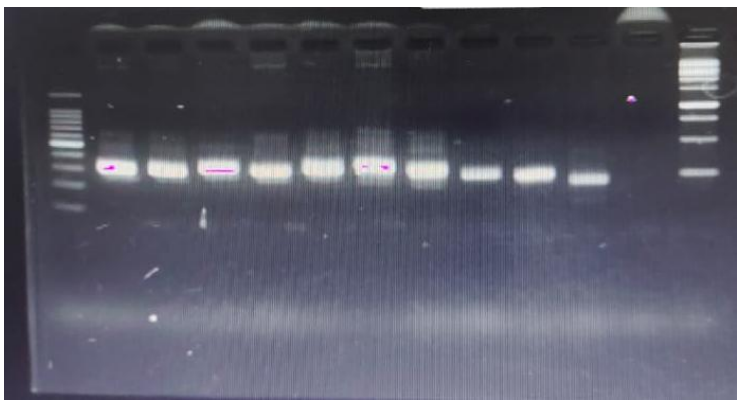


**DNA
assessment**

- 1
- 2
- 3
- 4**
- 5
- 6



Library preparation

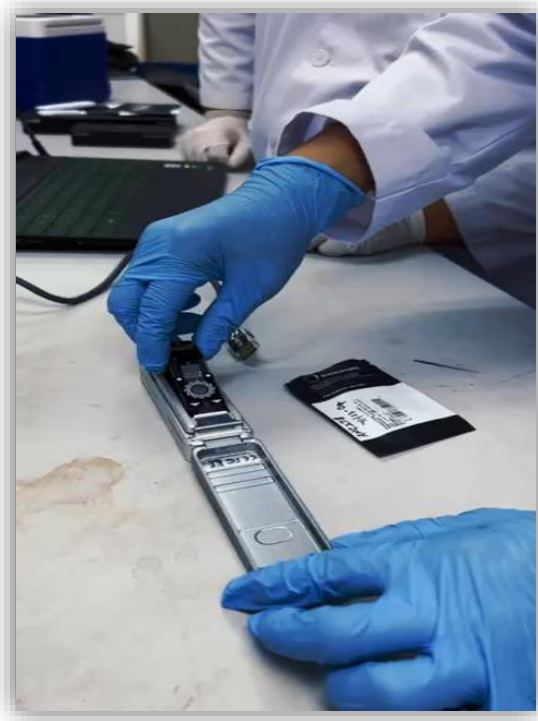


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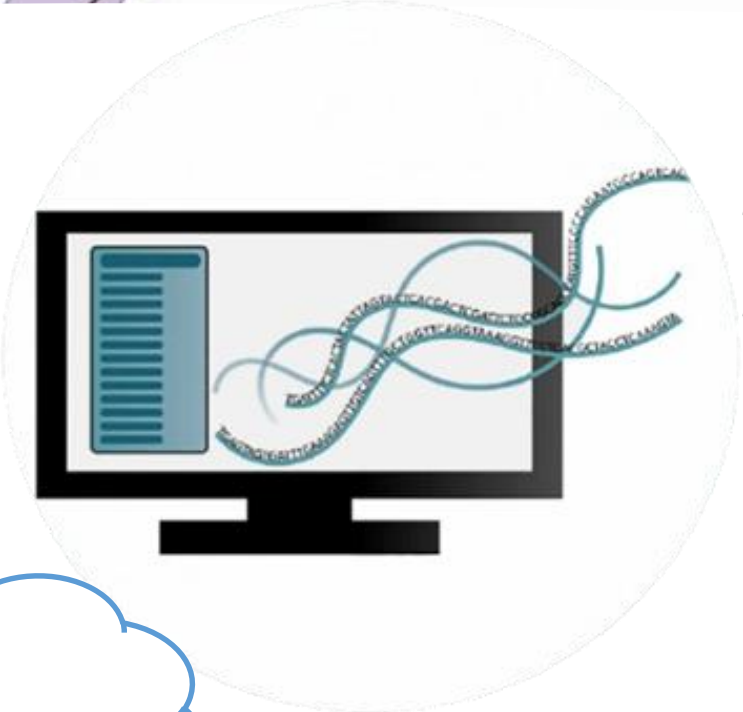




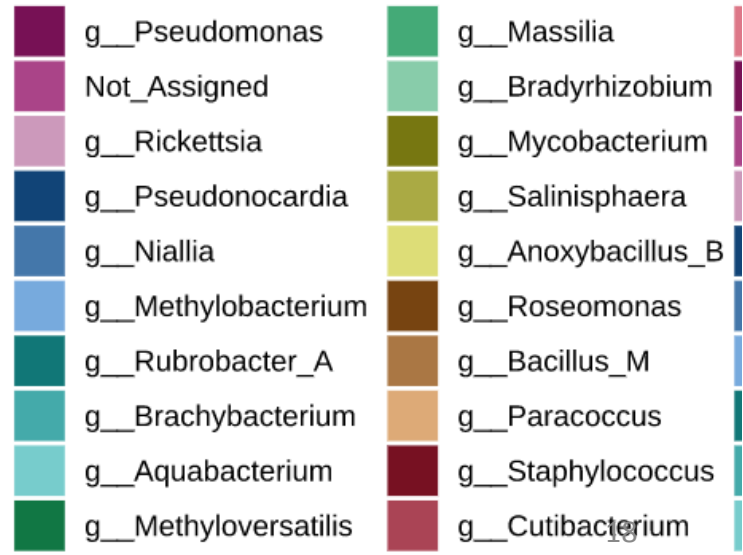
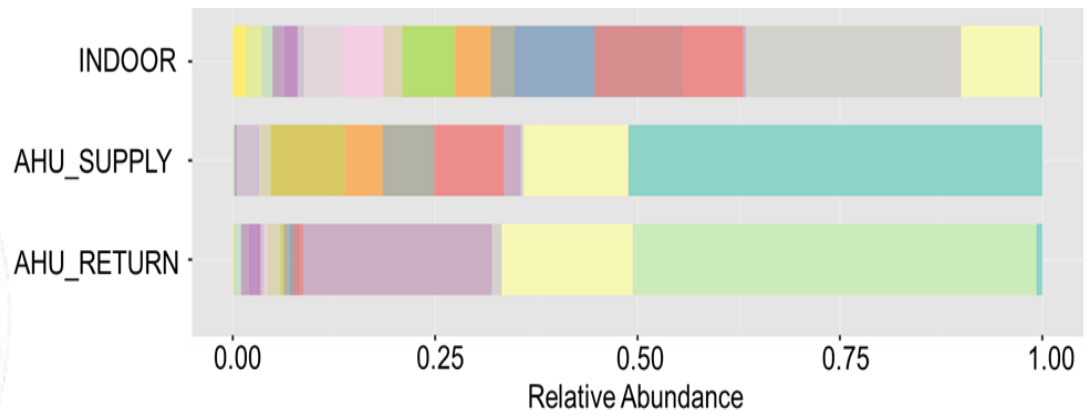
DNA sequencing



- 1
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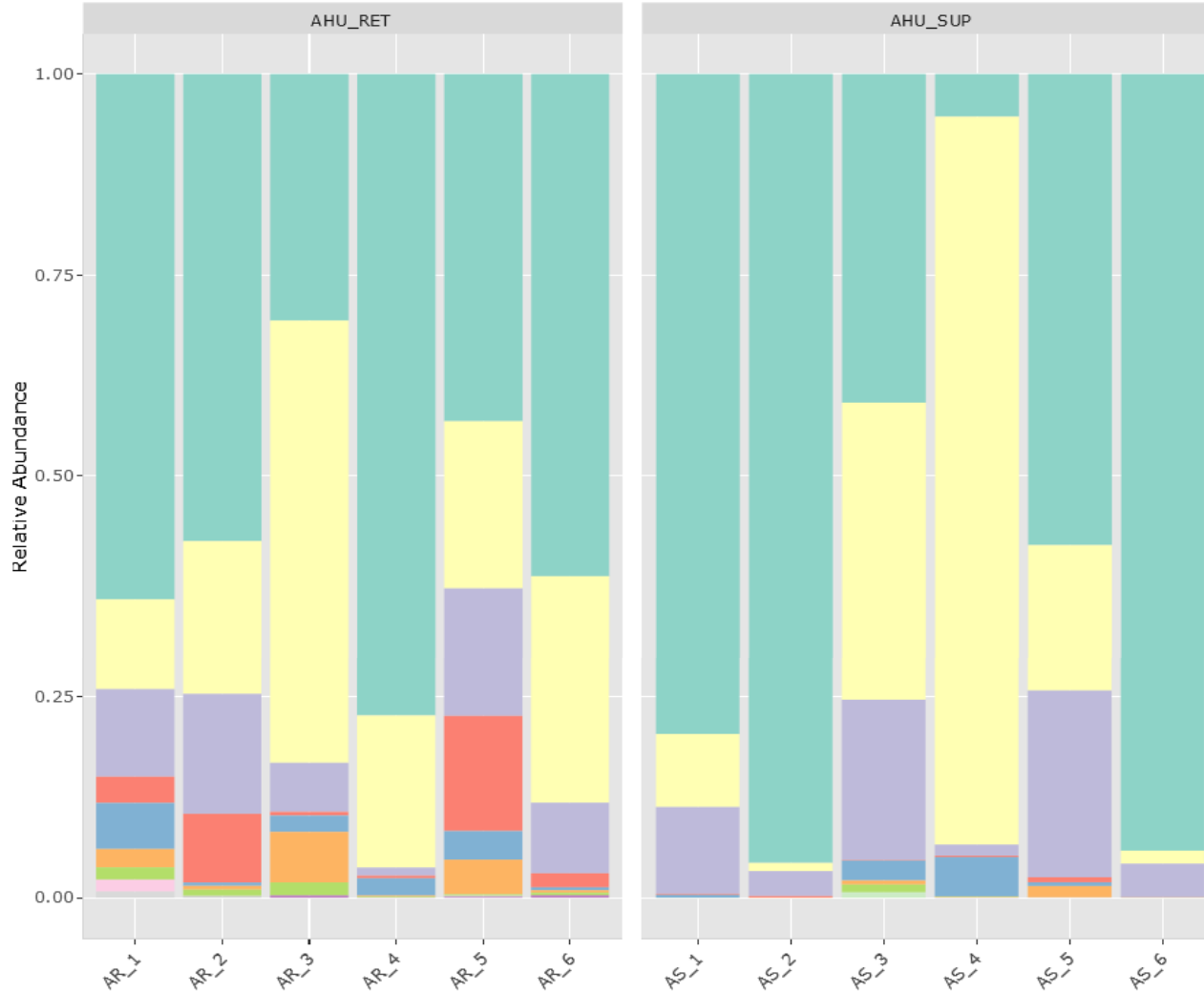


Bioinformatics



Phylum

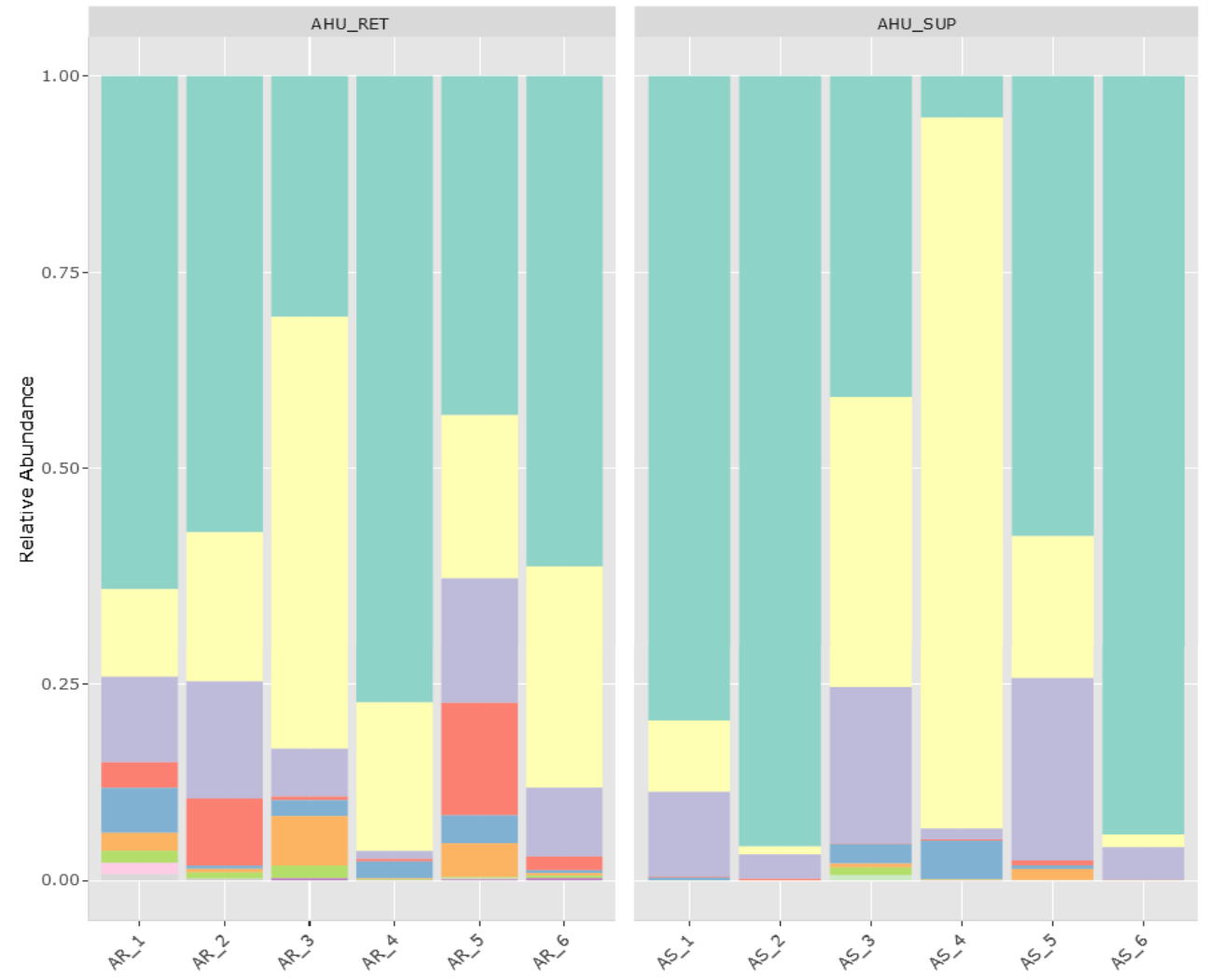
- p__Proteobacteria
- p__Actinobacteriota
- p__Firmicutes
- p__Chloroflexota
- p__Bacteroidota
- p__Cyanobacteria
- p__Myxococcota
- p__Planctomycetota
- p__Acidobacteriota
- p__Deinococcota
- p__Patescibacteria





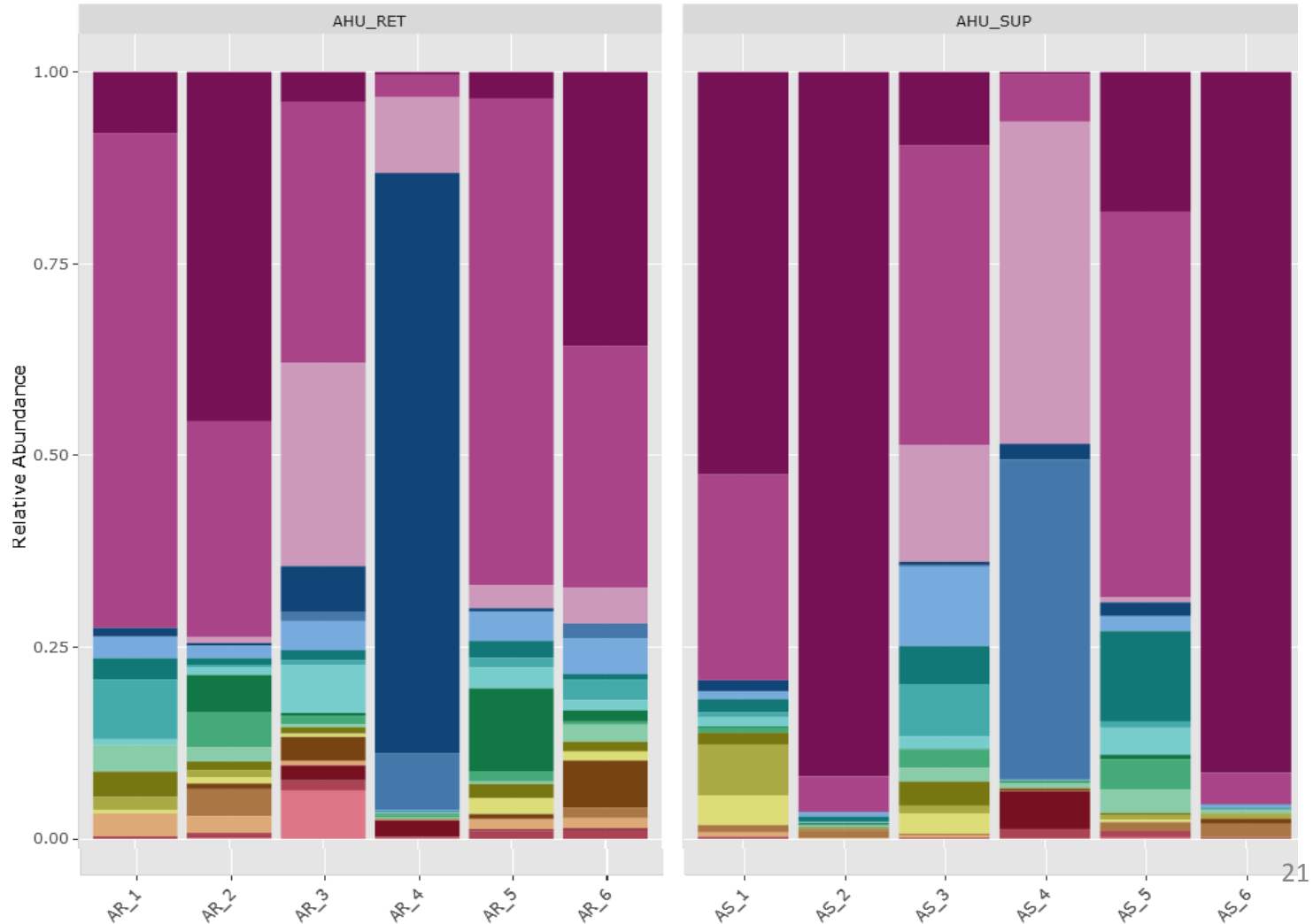
Phylum

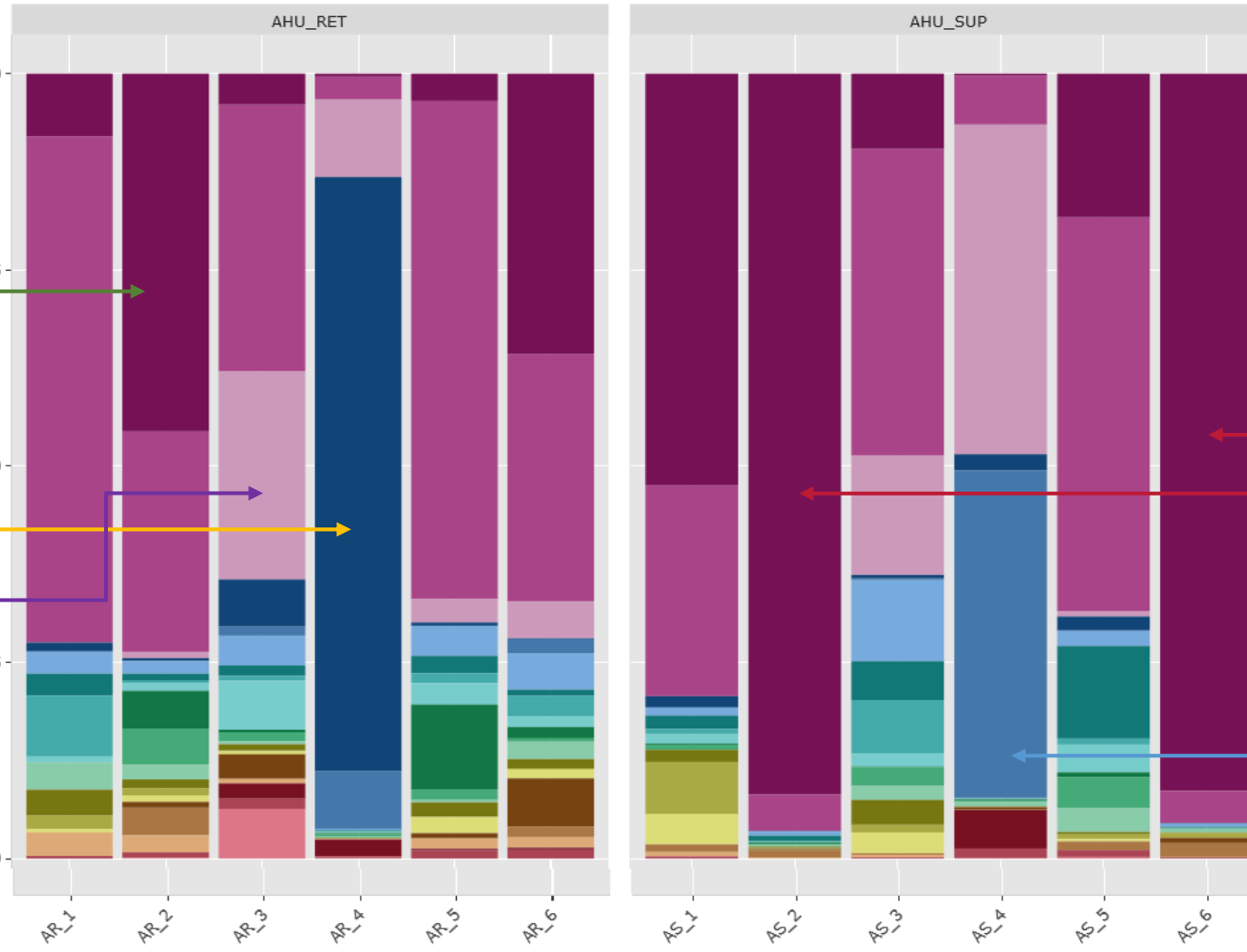
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- p__Actinobacteriota
- p__Firmicutes



Species

- g(U)_Methylobacterium
- Others
- s__Rubrobacter_A_bracarensis
- g(U)_Salinisphaera
- g(U)_Nesterenkonia
- f(U)_Sphingomonadaceae
- g(U)_Priestia
- f(U)_Caulobacteraceae
- f(U)_Acetobacteraceae
- f(U)_Ktedonobacteraceae
- g(U)_Pseudonocardia
- f(U)_Pseudomonadaceae
- s__Cutibacterium_acnes
- f(U)_Enterobacteriaceae_A
- f(U)_Micrococcaceae
- s__Rubrobacter_A_aplysinae
- f(U)_Planococcaceae
- g(U)_Staphylococcus
- f(U)_Flavobacteriaceae
- c(U)_Actinomycetia
- g(U)_Rubrobacter_A





Methylobacterium spp.

Salinisphaera spp.

Rubrobacter braccarensis.

Methylobacterium spp.

Nesterenkonia spp.

DISCUSSION

This study venture into **new culture-independent method** in **profiling of microbial community-**
new insight on the characterization and **fill gaps on microbes that are not culturable**

Bacterial DNA can be extracted and analyse directly from the **environmental samples** without the necessity to culture and grow the bacteria in the laboratory-
rapid analysis for a **large amount of samples-** necessity in **pathogens detection during outbreak**

Methylobacterium spp.,
Nesterenkonia spp., *Rubrobacter A bracarensis*, and *Salinisphaera* spp.

Most isolated species known as **opportunistic pathogens-** immunocompromised px, no significant effect on HCW



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CONCLUSION

Culture-independent method of sampling

Amplicon sequencing and next-generation sequencing (NGS)

Comprehensive profiling of microbial community indoors in a short period with high sensitivity and specificity



THANK YOU..

