Project No. 848228

DISCOvERIE

Development, dIagnostic and prevention of gender-related Somatic and mental COmorbitiEs in iRritable bowel syndrome In Europe

Workpackage 5
Deliverable D5.2

Report on microbiome and metabolome profiling in IBS and comorbid IBS

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17	Cliclab Technologies Inc	ES	Cliclab
18	Followhealth SL	ES	hITc
19	BYTE Factory Systems SL	ES	BF

General hints for writing a deliverable:

List of abbreviations

FGFP: Flemish gut flora project
BL: Baseline
FU1: First follow-up (after one year)

Discoverie Glossary

Executive Summary

Deliverable D5.2 describes the current status of the metagenomic and metabolomic data generated in the WP5. The data generated includes total cell count quantification, faecal moisture measurement, shotgun sequencing, and metabolome profiling. Despite logistical challenges and technical delays, the metabolome, microbiome, the cell counts, and the moisture have been completed.

Introduction

Irritable Bowel Syndrome (IBS) is a chronic gastrointestinal disorder characterized by abdominal discomfort and irregular bowel movements, which can include constipation and/or diarrhoea. Despite its prevalence, the exact causes of IBS are not fully understood, but it is believed to involve various factors, including the gut microbiota. However, current research primarily relies on 16S rRNA amplicon sequencing, which provides a broad overview of the bacterial composition but needs more detailed insight into the functional potential of the gut microbiome. To address this gap, WP5 will conduct shotgun sequencing and metabolomics analyses on faecal samples collected from participants in the DISCOvERIE cohort, gathered from nine different collection centres. The report shows the metagenomic and metabolomic data generated from the samples obtained from deliverable D5.1(Report on faecal sampling and transport of IBS cohorts in the project and the quantitative microbiome profiling)

Report on cell count quantification

The total cell count quantification has been completed for the 841 samples received, covering all the baseline samples of the cohort. The total cell count quantification provides valuable insight into the overall bacterial load, enabling a more accurate estimation of the abundance differences among bacterial taxa.
The cell quantification was obtained via flow cytometry. Briefly, frozen (-80°C) faecal samples weighing 200-250 mg were diluted in a saline solution (0.85% NaCl; VWR) and then filtered through a sterile syringe filter (pore size: 5 µm). Subsequently, 1 mL of the microbial cell suspension underwent staining with 1 µL of SYBR Green I (diluted at a ratio of 1:100 in DMSO, sourced from Thermo Fisher Scientific in Massachusetts, USA), followed by a 15-minute incubation in darkness at 37°C. Flow cytometry analysis uses a C6 Accuri flow cytometer from BD Biosciences in New Jersey, USA. Fluorescence events were observed using optical detectors with FL1 533/30 nm specifications and FL3 >670

nm. The BD Accuri CFlow software was employed to define and distinguish microbial fluorescence events on the FL1/FL3 density plot, differentiating them from the background of the faecal sample. A threshold value of 2000 was set for the FL1 channel. Cell counts are converted into microbial loads per gram of faecal material based on the precise weight of the analyzed aliquots.

The total cell count quantification showed that the DISCOVERIE cohort has a significantly higher cell count quantification than the FGFP cohort, a large cohort population cohort of Flemish subjects (Figure 1A).

Report on moisture measurement

The faecal moisture quantification has been completed for the 841 samples received, covering all the baseline samples of the cohort. The faecal moisture is a proxy for transit time, a significant determinant of gut microbiome composition. Estimating the transit time in microbiome analyses is crucial to differentiate genuine ecological shifts or abundance variations attributable to a specific disease or condition from normal microbiome variation. The faecal moisture content was calculated by assessing the percentage of mass reduction following lyophilization of 0.2 grams of frozen, non-homogenized faecal material.

Similarly to what was observed for the total cell counts, the faecal moisture is significantly higher than what it is observed in the Flemish population (FGFP cohort) (Figure 1B)

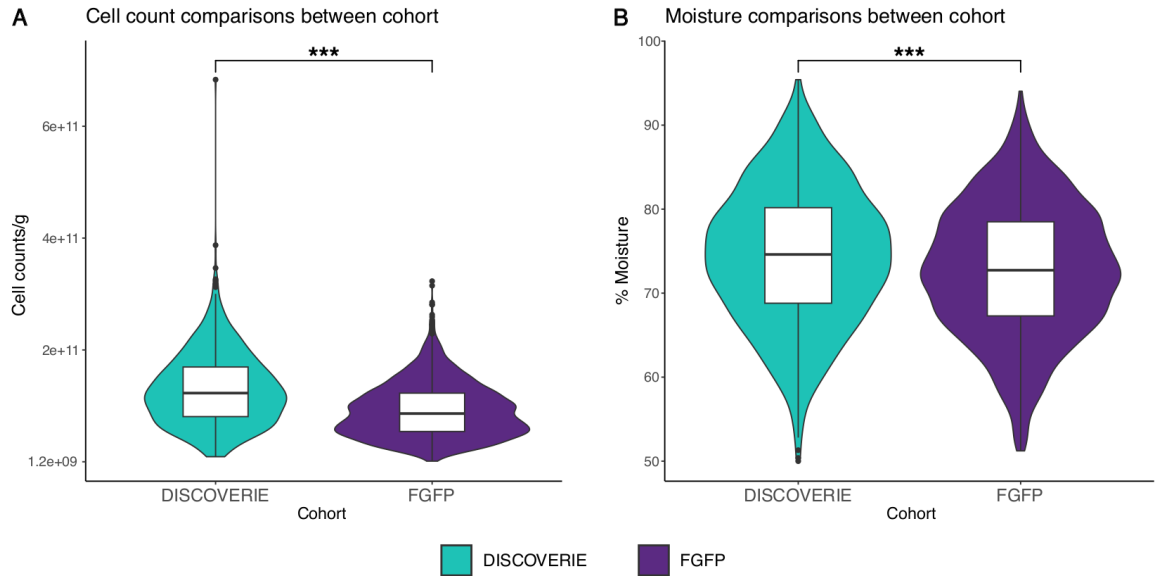

Figure 1: differences in cell counts per gram of faecal matter (A) and moisture (B) between the DISCOVERIE and the FGFP cohort

Report on shotgun sequencing

The faecal DNA was extracted, and the metagenomic library construction was completed for the 841 samples received. The DNA was extracted from 150-200mg of the frozen samples using MagAttract PowerMicrobiome DNA/RNA KF kit (QIAGEN) following the manufacturer's instructions. The library construction was done using Illumina Novaseq commercial kits.

841 samples were sent for sequencing, for which the large majority yielded successful results (83%), which is sufficient for succesfully completing the project. The average

sequencing coverage per sample was 5.494±3.716gb, allowing us to estimate the taxonomic and functional composition of the most dominant taxa of the bacterial composition.
The Raes' lab have developed different bioinformatic and statistical pipelines to analyse the metagenomic dataset and its further multiomic integration (Figure 2).
Additional analysis focusing on function quantification and varying taxonomic resolutions will be conducted based on the specific biological questions pertinent to the analysis, which will be determined as the data from different work packages intersect and accumulate.
Despite delays in the samples' shipments (Details in report D5.1),sufficient samples received from the baseline condition have been sequenced. Currently, we are working on the data analysis for the sequenced samples.

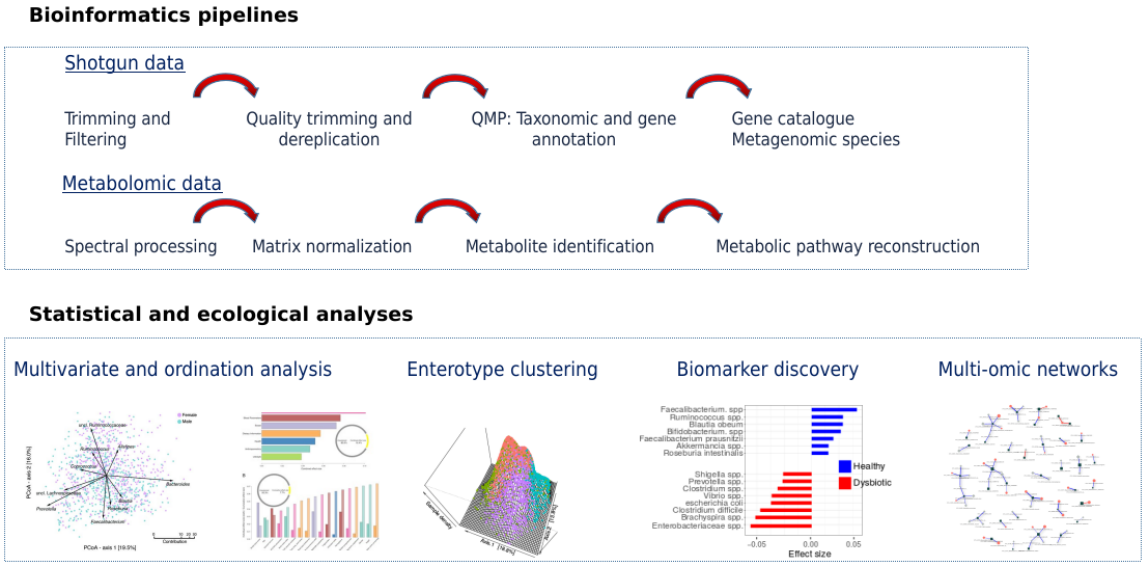

Figure 2: diagram of the bioinformatics and statistical analysis for the multiple omics data generated in the work package 5

Report on the metabolome profiling

The metabolomic profiling has been completed for 840 (all baseline samples and 87 for the follow-up) samples of the DISCOVERIE cohort. The change of the report delivery date to 28/02/2024 was given due to delays in sample delivery, as detailed in T5.1, and the installation and repair of a module on the Scimax and Lumos platforms at the VIB metabolomics core. The delays in the VIB metabolomic core facilities compelled us to find an alternative provider for performing the metabolomic profiling.
As mentioned in the subcontracting activity Justification Letter, the VIB contacted four companies to comply with the financial rules set for subcontracting activities.
- Biocrates;
- Clinical Microbiomics AS;
- General Metabolomics;
- Metabolon.

The service requested was to perform:
- Metabolic profiling on the provided sample extracts
- Quality control and data standardization

Based on the quality of the service, the price per sample and the time from which they can generate the results, we selected **General Metabolomics** (https://generalmetabolics.com/) to perform this subcontracting activity.
Following the company's recommendations, the metabolites from the faecal samples, such as central sugars and sugar phosphates, nucleotides, organic acids, amino acids, and some of the less polar fatty acids as polar fatty acids, were extracted with 80% methanol / 20% from frozen (-80ºC) samples. The samples were shipped to General Metabolics, who performed a high-resolution, non-targeted metabolomics approach for profiling the sample extracts.
The profiling showed 986 metabolites present in the faecal samples, where we identified many of them as relevant for gut-brain axis communication and might play a role in the maintenance or development of the IBS (Figure 3A). Moreover, the sample distribution showed a clustered configuration, indicating different profiles in the metabolic potential of the bacterial communities (Figure 3B). Interestingly, the total cell count and the faecal moisture content significantly explained 2.2% of the variation in the metabolic content of the bacterial population (Figure 3C). Further analysis will include the multi-omic integration with the data layers generated in the cohort (Figure 2), and will be guided by results from other Work Packages.

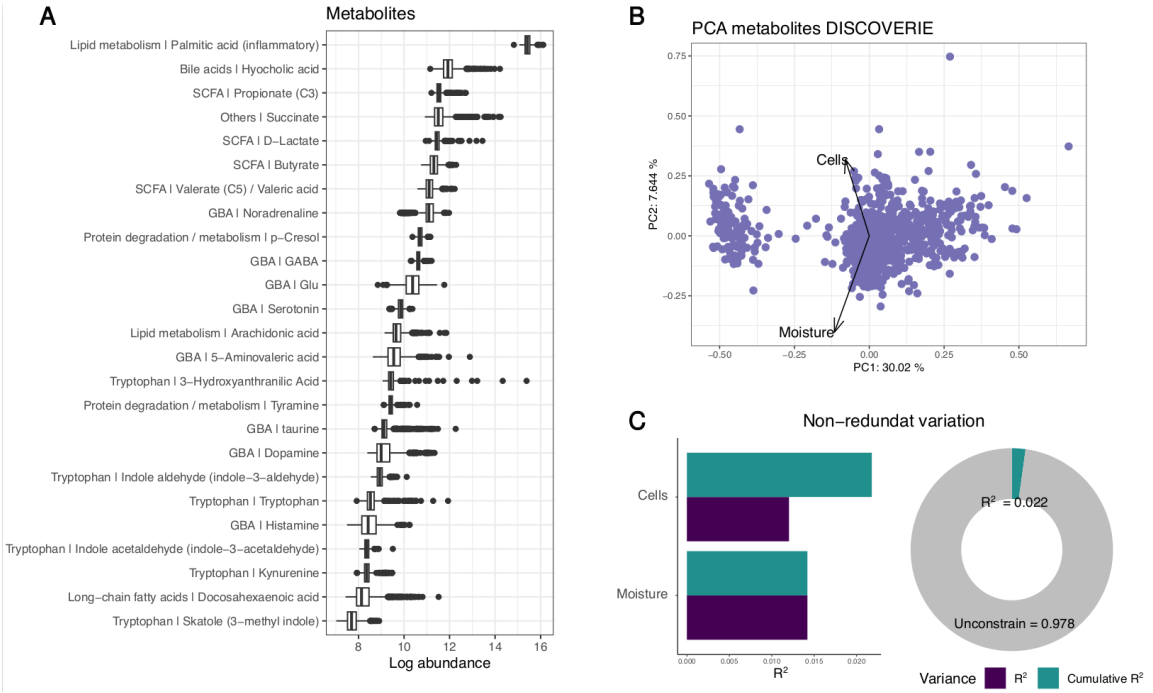

Figure 3: Metabolome features and variation. (A) Key metabolites involved in gut-associated dysbiosis and the gut-brain axis. (B) Metabolome Principal Coordinate Analysis. (C) Non-redundant variation explained by the faecal moisture and the total bacterial cell counts

Conclusions

Comprehensive analyses have been conducted on the DISCOVERIE cohort samples, including total cell count quantification, faecal moisture measurement, shotgun sequencing, and metabolome profiling. Delays in deliverables and a new report date of 28/02/2024 were given due to delays in the sample shipments and technical problems with installing the Scimax metabolomic platform on time.

Currently, the baseline samples have undergone cell count quantification, moisture estimation, shotgun metagenomics and metabolome profiling.

The multi-omic data generated will be crucial for further exploration of microbial-host interactions and the underlying mechanisms in the IBS gut-associated bacterial communities. Future analysis using the data from the different work packages will elucidate the complex relationships that influence IBS development.