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Multi-omics approach in gut and environmental microbiota research under the One Health concept

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Abstract

The One Health concept, although formulated two decades ago, remains challenging to implement. It necessitates the integration of numerous scientific disciplines, diverse techniques and various professional expertise. Furthermore, it often requires the collaboration of different institutions, encompassing both scientific and administrative entities. This concept posits that human health is intrinsically linked to and dependent on the well-being of animals, plants and the broader environment, while the environment not only sustains life but can also serve as a source of xenobiotics that affect the health-disease balance. In this context, all components of the potential exposome, encompassing the entirety of factors of various natures that influence health throughout life, must be considered comprehensively. Achieving this holistic understanding typically demands the application of multiple research techniques, known as the multi-omics approach and the adoption of an integrated method for data analysis. This project endeavoured to utilise such an integrated approach, examining data from diverse origins: human (children stool for gut microbiota analysis) and environmental (groundwater for hyporheic zone microbial analysis), as well as implementing comprehensive informatic tools for data processing. Analysis of stool samples revealed significant differences in gut microbiota composition across various taxonomic levels between normal weight, overweight and obese children. Additionally, a potential link between certain xenobiotics and gut microbiota composition, body weight and overall health status was identified. Analysis of groundwater samples revealed significant differences in hyporheic zone microbial composition at various taxonomic levels based on the sampling location and depth. Key geochemical factors influencing sample diversity were also identified. The promising results obtained not only demonstrate the viability of this methodology but also pave the way for future research initiatives.

KEYWORDS

environmental health, exposome, human health, microbiota, xenobiotics

CONTENTS

1 | INTRODUCTION

The concept of One Health is defined as 'an integrated and unifying approach to sustainably balance and optimise the health of people, animals and ecosystems' (OHHLEP et al., 2023). It aims to balance and optimise the health of people, animals, plants and the environment, acknowledging their interdependencies. This term was first used in 2003–2004 in response to the emergence and spread of two highly pathogenic viruses: SARS (severe acute respiratory syndrome) and H5N1 (avian influenza). These events clearly demonstrated that human health is inseparable from the health of animals and the broader environment, initiating cross-sector collaboration among institutions such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), World Organization for Animal Health (OIE), United Nations Children's Fund (UNICEF), United Nations System Influenza Coordination (UNSIC) and the World Bank. This collaboration relies on effective governance, communication and coordination. One Health addresses health challenges such as infectious diseases, antimicrobial resistance and food safety, while promoting ecosystem sustainability and integrity. This approach supports disease control from prevention to response and enhances global health security, helping stakeholders understand the benefits, risks and opportunities for holistic solutions (Mackenzie & Jeggo, 2019).

One aspect of human health that has attracted particular interest in recent years is the intestinal environment, especially the gut microorganisms (viruses, archaea, bacteria, fungi, protozoa), called conjointly gut microbiota (Berg et al., 2020), while gut microbiome refers to specific microbial genes, proteins and enzymes (Lahti & Shetty, 2012–2019). Since 1992, when the human intestinal microbiota was referred to as a 'neglected organ' (Bocci, 1992), its role is now far more recognised than initially believed to simply improve digestion through processes like the fermentation of non-digestible substrates such as dietary fibres and endogenous intestinal mucus. The gut microbiota is now considered a metabolising organ capable of producing and regulating key enzymes, metabolites, vitamins and hormones, and playing an essential role in physiological and clinical states, illnesses and metabolic disorders such as obesity, diabetes, cardiovascular diseases and others (Torres-Sánchez et al., 2023). Therefore, it is often called the 'second liver' (Lindell et al., 2022). Moreover, the role of microbiota in mental disorders such as anxiety, depression or bipolar disorder is also well established, leading to it being termed the 'second brain' (Cryan et al., 2019; Gershon, 1998) for which molecular mechanisms are starting to be elucidated (Cerdó et al., 2023). Additionally, the number of genes carried by intestinal microorganisms is many times greater than the number of host genes (Gilbert et al., 2018), which is why the microbiota is also referred to as the 'second genome' (Grice & Segre, 2012).

The modern understanding of human health, in relation to the well-being of animals, plants and the entire environment, has led to the creation of the exposome concept, first defined in 2005 (Wild, 2005), supporting the main principles of the One Health idea. The exposome encompasses all the factors to which a person is exposed from conception, through development in the mother's womb, to death – essentially throughout one's entire life. By considering the influence of environmental factors (including lifestyle factors), the exposome complements the genome (Siroux et al., 2016; Vermeulen et al., 2020). Exposome analysis is a complex task that requires collaboration across various scientific disciplines (Siroux et al., 2016), such as microbiology, nutrition, toxicology, analytical chemistry, food safety and personalised medicine. Additionally, achieving the desired results necessitates the integration of multiple health communities, professional fields and technologies, with artificial intelligence-based tools playing an increasingly important role (Merino Martinez et al., 2021; Ortiz et al., 2022). The multi-omics approach, which employs several techniques such as genomics, transcriptomics, proteomics, metabolomics, lipidomics, epigenomics and others, is crucial in elucidating the mechanisms behind many diseases, disorders and dysbiosis caused by dietary exposure to toxic compounds (Gruszecka-Kosowska, Ampatzoglou, & Aguilera, 2022; Gruszecka-Kosowska, Ampatzoglou, & Aguilera-Gómez, 2022). The One Health approach and the concept of the exposome are also important elements of, among others, Next Generation Risk Assessment (Ampatzoglou et al., 2022) and Next Generation Probiotics (Torres-Sánchez et al., 2022).

The other remarkably important component of the One Health concept is the environment, which hosts and feeds us but is also a potential source of xenobiotics that contaminate the air (EEA, 2024; Munir et al., 2019; Strzebońska et al., 2020), soil (Carré et al., 2017; Guo et al., 2014), sediments (Heim & Schwarzbauer, 2013; Kostka & Leśniak, 2021) and water (Strzebońska & Kostka, 2021; Tsakiris, 2015), thus affecting human health. The multi-omics approach or selection of omics data, though primarily applied in human health research, is also utilised in environmental studies, particularly for investigating microbial communities across various ecological niches (McDaniel et al., 2021; Shaffer et al., 2022). As part of the fellowship programme, a multi-omics methodology was applied to process two distinct sample groups: human (stool for gut microbiota analysis) and environmental (groundwater for hyporheic zone microbial analysis). This integrative approach not only underscores the interconnectedness of human and environmental health but also advances our understanding of the complex interplay between these domains, transferring methodologies, paving the way for more holistic and effective strategies in health management and environmental conservation.

2 | DESCRIPTION OF WORK PROGRAMME

2.1 | Aims

The work programme described below was carried out in alignment with the legal framework of the EUropean FOod Risk Assessment (EU-FORA) Fellowship Programme, under the title 'Microbiome exposome analysis for risk assessment of xenobiotics exposure and the impact on human gut dysbiosis – One Health concept (MICROEXP-ONEHEALTH)'. Work programme was proceeded, according to the following objectives:

- **OBJECTIVE 1**. To learn main available methods and omics technologies for exposome and microbiota analysis (composition/activity patterns) while exposed to different level of diet hazardous substances cumulative in the environment.
- **OBJECTIVE 2**. To obtain upmost information about exposome and human microbiota variability, bioresources and dysbiosis associated and/or putatively caused by diet hazardous substances environmental and consumption exposures.

2.2 | Data and Methodologies

2.2.1 | Sampling, data collection and methods

The programme's objectives referring to practical part of the fellowship were implemented based on two sets of data:

- **Stool samples** from the faecal collection of the hosting institution (University of Granada, Spain), initially collected from children participating in the OBEMIRISK project (Aguilera et al., 2022). Samples were taken using an in-house anaerobic kit, immediately frozen at −20°C, then transferred into −80°C and kept until further analysis.
- **Environmental samples (groundwater)** from the sending institution (AGH University of Krakow, Poland), obtained from the research project of the National Science Centre of Poland, titled 'Geomorphological, hydrogeological and hydrochemical criteria for assessing the functioning of the hyporheic zone of polluted rivers'. Samples were taken from previously installed piezometers. At least three volumes of water were purged from the piezometers before sampling using low-rate submersible pump, then samples were filtered and kept refrigerated until further analysis (Aleksander-Kwaterczak & Ciszewski, 2016; Ciszewski, 2019; Ciszewski & Aleksander-Kwaterczak, 2016; Ciszewski & Bijata, 2015).

Further analysis of stool samples included culturomics, metagenomics, chemical analysis and metadata collection. Isolation of gut bacteria was carried out in anaerobic conditions using Work-Anaerobic Stations (Don Whitley Scientific, Bingley, Great Britain) and Anaerocult® System (Merck, Darmstadt, Germany), with several culture media such as BHI (Brain Heart Infusion Agar), MRS (Man–Rogosa–Sharpe Agar) and others. Final identification of isolated strains was based on the MALDI–TOF-Biotyper Sirius-Bruker facilities (courtesy of University Hospital San Cecilio, Granada, Spain), and (if necessary or applicable) on Sanger 16S rRNA gene sequencing, based on DNA obtained from pure cultures using DNeasy columns (Qiagen®, Hilden, Germany). Metagenomic studies were performed through 16S rRNA gene analysis. For this purpose, DNA was extracted from stool samples using the PowerSoil DNA Isolation Kit (Qiagen®, Hilden, Germany), followed by sequencing of the V4 hypervariable region of the 16S rRNA gene using Illumina MiSeq Platform (Novogene Sequencing – Europe) (López-Moreno et al., 2023; Ruiz et al., 2017). As a result of chemical analysis, concentrations of selected xenobiotics (i.e. bisphenol A – BPA, bisphenol S – BPS, bisphenol AF – BPAF, methylparaben – MetPB and ethylparaben – EthPB) were determined in children hair samples, as previously described (Moscoso-Ruiz et al., 2022; Rodríguez-Gómez et al., 2017). Metadata included anthropometric measures (i.e. sex, age, BMI, height, weight, waist circumference, hip circumference, fat content, muscle content and water content) which were performed according to the guidelines of the World Health Organization (de Onis et al., 2007).

Further analysis of groundwater samples included metagenomics, geochemical analysis and metadata collection. DNA for metagenomics purposes was extracted from samples according to Sherlock AX and Genomic Mini AX Bacteria procedures (A&A Biotechnology, Gdańsk, Poland), followed by sequencing of the V3-V4 hypervariable region of 16S rRNA gene, performed with Illumina MiSeq v2 Reagent Kit (GENOMED, Warsaw, Poland). Geochemical analysis included determination of basic parameters such as pH, conductivity and oxygen content, which were measured in situ using a Thermo Scientific-meter. Anions (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, CO_3^{2-}) and macro-cations (Ca, Mg, Na, K) were determined using ion chromatography (DIONEX 1000), while other elements (As, B, Ba, Bi, Cd, Cs, Fe, Ga, Mn, Mo, Ni, Pb, Rb, S, Sb, Si, Sn, Sr, Tl, V, Zn and Zr) were measured with an inductively coupled plasma-mass spectrometer (Perkin Elmer ELAN 6100) at the certified laboratory (Aleksander-Kwaterczak & Ciszewski, 2016; Ciszewski, 2019; Ciszewski & Aleksander-Kwaterczak, 2016; Ciszewski & Bijata, 2015), following the standard certified analytical quality control procedure (PN-EN ISO 17294-1, 2007). Appropriate standard reference materials were used to ensure the accuracy of analysis. Metadata included details of sampling sites (location, depth, distance from riverbed and season).

2.2.2 | Data processing

The integration of different types of data (metagenomics, culturomics, metabolomics, chemical data, metadata, etc.) is challenging and requires a comprehensive approach and standardisation (Chetty & Blekhman, 2024; Gruszecka-Kosowska, Ampatzoglou, & Aguilera, 2022). This necessitates the use of increasingly sophisticated tools that are often dedicated to specific types of data, particularly 16S rRNA gene sequencing results and the accompanying metadata (Xu et al., 2023). To achieve this goal, the fellowship holder completed two preparatory workshops, applying such tools as QIIME2 (Bolyen et al., 2019) and Galaxy (The Galaxy Community, 2024):

- 2023.11.17–18: workshop 'Bioinformatics for biologists – using data from high-throughput sequencing (NGS) to analyse the composition of microbiomes' (realised at the sending institution).
- 2023.12.01–02: workshop 'Bioinformatics for biologists – using the GALAXY tool to identify variants using data from high-throughput NGS sequencing' (realised at the sending institution).

The core part of the fellowship, fulfilling its practical aspects, was an intensive R software training and in-house learning course conducted at the hosting institution from January to June 2024 (Alicia Ruiz-Rodríguez and Ana López-Moreno). This course specifically included 16S rRNA gene data processing and filtering, data association, statistical analysis and data visualisation, following the best practices in microbiome analysis using R (Wen et al., 2023). In particular, professional R software tools and packages dedicated to processing 16S rRNA gene sequencing results, such as *Biostrings* (Pagès et al., 2013), *metagenomeSeq* (Kim, 2019), *metagMisc* (Mikryukov, 2017), *microbiome* (Lahti & Shetty, 2012–2019), *microViz* (Barnett et al., 2021) and *phyloseq* (McMurdie & Holmes, 2013), were used. Additional packages included, among others, *Bioconductor* (Callahan et al., 2016), *dplyr* (Wickham, François, et al., 2019), *ggplot2* (Wickham, 2009), *tidyverse* (Wickham, Averick, et al., 2019) and *vegan* (Dixon, 2003).

2.3 | Additional activities

During the fellowship period, Anna Kostka, as a fellowship holder, participated in five mandatory EU-FORA training modules, organised by the European Food Safety Agency (EFSA), in a cooperation with other European institutions, including the Austrian Agency for Health and Food Safety (AGES), the German Federal Institute for Risk Assessment (BfR), the Hellenic Food Authority (EFET) and SAFOSO (Swiss consultancy, research and capacity-building company). Anna Kostka also participated in a visit to the Spanish Agency for Food Safety and Nutrition (AESAN) in Madrid (Spain) (February 27–28, 2024). Additionally, she attended several conferences, webinars, training sessions and science divulgation, aligned with her personal and professional interests.

Webinars, courses and science divulgation:

- 2023.11.22, Krakow (Poland): training 'The role of scientific journals in the development of a scientist's career'
- 2024.01.11, CIBM, UGR (Granada): INYTA Women in Science Scientific Divulgation Video, https://youtu.be/G1tcbngCSkU
- 2024.03.13, online: webinar 'Next-generation metagenomics of the human microbiome: from bench to bedside'
- 2024.03.14, online: webinar 'Big data meets reductionist experimentation: A synthetic fecal transplant clarifies resistance to *C. difficile* infection'
- 2024.03.21, online: webinar 'Exploring the Microbial World: A Journey through Sequencing Applications'
- 2024.06.14, online: webinar 'Green Open Access – how to be a scientist visible on the web'
- 2024.07.23, online: webinar 'Long-read sequencing, the next era of genomics'
- INYTA News – International Collaborations, https://inyta.ugr.es/instituto/noticias/colaboraciones-entidades-internacionales

Communication to Congresses:

- 2024.02.29, Brussels (Belgium) and online: 'Human Microbiome Action – Final Conference' (Kostka et al., 2024)
- 2024.06.07, online: '8th National Polish Microbiological Scientific Conference MICROBS' (manuscript currently under review)
- 2024.06.19–21, Granada (Spain): 'XXXIII Congress of the Spanish Society of Nutrition (SEÑ)' (Aguilera, Ortiz, et al., 2024)

3 | OUTCOMES

3.1 | Preliminary results of stool samples and groundwater samples analysis

Advanced data investigation methods using R software allowed for a comprehensive statistical analysis of two datasets (stool samples for gut microbiota analysis and groundwater samples for hyporheic zone microbial analysis), integrating information obtained from different sources and techniques (metagenomics, metabolomics, chemical analysis, metadata), searching for patterns, connections and correlations. Data were analysed and visualised using various tools and methods

such as relative abundance, alpha-diversity measures, beta-diversity measures, non-metric multi-dimensional scaling (NMDS), permutational multivariate analysis of variance (PERMANOVA), correlations between environmental variables and ordination, heatmaps and some others.

3.1.1 | Omics metrics of stool samples

Analysis of stool samples showed significant differences in the composition of gut microbiota at various taxonomic levels between normal weight (NW), overweight (OW) and obese (O) children. A potential relationship between some xenobiotics and the composition of individual intestinal microbiota, as well as with body weight and overall health status, was also demonstrated. These preliminary results stay in line with previous research of the BIO-190 Group at the University of Granada (López-Moreno, Acuña, et al., 2021; Lopez-Moreno et al., 2024; López-Moreno et al., 2023; López-Moreno et al., 2024; López-Moreno, Torres-Sánchez, et al., 2021), however require further investigation. Related studies conducted on the same group of children (Salcedo-Bellido et al., 2024) and some other papers (e.g. Bist & Choundhary, 2022; Vaccari et al., 2023) have also shown a potential association between obesogenic conditions and other xenobiotics, namely metals. The analyses were based on the concentration of selected metals in urine obtained by researchers of the University of Granada (Salcedo-Bellido et al., 2024), however, stool samples were not investigated. Therefore, the hosting institution, in cooperation with the fellowship holder, prepared faecal samples for analysis of the presence and concentration of metals, which are currently being carried out at the sending institution.

3.1.2 | Omics metrics of groundwater samples

Analysis of groundwater samples showed significant differences in the composition of hyporheic zone microbial communities at various taxonomic levels depending on the location and depth of the sampling site. Key geochemical factors driving sample diversity were also identified. The obtained results are consistent with previous studies (Aleksander-Kwaterczak & Ciszewski, 2016; Ciszewski, 2019; Ciszewski & Aleksander-Kwaterczak, 2016; Ciszewski & Bijata, 2015), which were however based solely on geochemical and hydrochemical parameters. Therefore, current findings complement and enhance the understanding of the hyporheic zone environment, and further research will continue.

3.2 | Literature overview

Another aspect of the fellowship work programme was to gather comprehensive information about the exposome and human gut microbiota variability. This was accomplished through an extensive literature review using search engine databases (PubMed, SciFinder, Web of Science, Scopus, Embase) in accordance with the guidelines of the European Food Safety Authority (EFSA, 2015). In pursuit of this goal, the fellowship holder prepared a thorough literature study on the history of research on human gut microbiota, which is planned for future publication. It covers topics such as: (1) the discovery of microorganisms and 'germ theory'; (2) the beginnings of human microbiota studies; (3) the concepts of 'bad germs' and 'good germs' (infectious microorganisms, the theory of autointoxication, probiotics prebiotics and synbiotics, faecal transfers); (4) modern techniques in microbiota studies (anaerobic culturing, germ-free animal models, molecular revolution – DNA sequencing).

As a result of completing the theoretical part of the fellowship programme, three publications were released:

- poster communication from the 'Human Microbiome Action – Final Conference' titled 'Human microbiome become key informative for evaluating the impact of contaminants under the One Health concept' (Kostka et al., 2024).
- abstract communication from the 'XXXIII Congress of the Spanish Society of Nutrition (SEÑ)' titled 'Gut microbiome and omics metrics: Approaches for strengthening contaminant risk assessment and agri-food safety under the One Health' (Aguilera, Ortiz, et al., 2024).
- paper titled 'De la taxonomía descriptiva a la aplicada: Microbiota humana, la comunidad microbiana más diversa, mejor conectada y con mayor impacto en la salud' (Aguilera, López-Moreno, et al., 2024).

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