JOURNAL OF PROTEOMICS 97 (2014) 17–26

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

A metaproteomic pipeline to identify newborn mouse gut phylotypes☆,☆☆

Federica Del Chierico[a,b], Andrea Petrucca[a,b,c], Stefano Levi Mortera[d,e], Pamela Vernocchi[a,b,f], Maria M. Rosado[g], Luisa Pieroni[d,e], Rita Carsetti[g], Andrea Urbani[d,e,*,1], Lorenza Putignani[a,b,**,1]

[a]Parasitology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[b]Metagenomics Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[c]Department of Diagnostic Science, Sant'Andrea Hospital, Rome, Italy
[d]Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy
[e]IRCCS—Santa Lucia Foundation, Rome, Italy
[f]Interdipartimental Centre for Industrial Research, Alma Mater Studiorum, University of Bologna, Italy
[g]B-cell development Unit and Immunological Diagnosis Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

ARTICLE INFO

Available online 29 October 2013

Keywords:
Early life gut microbiota phylotypes
Mouse model
Metaproteomic pipeline
MALDI-TOF MS proteomics

ABSTRACT

In order to characterize newborn mouse gut microbiota phylotypes in very early-life stages, an original metaproteomic pipeline, based on LC–MS^2-spectra and Mascot driven NCBI non-redundant repository database interrogation was developed. An original computational analysis assisted in the generation of a taxonomic gut architecture from protein hits to operational taxonomic units (OTUs) and related functional categories. Regardless of the mouse's genetic background, a prevalence of Firmicutes (Lactobacillaceae) and Proteobacteria (Enterobacteriaceae) was observed among the entire Eubacteria taxonomic node. However, a higher abundance of Firmicutes was retrieved for Balb/c gut microbiota compared to $Rag2^{ko}$ mice, the latter was mainly characterized by a Proteobacteria enriched microbiota. The metaproteomic-obtained OTUs were supported, for the identification (ID) of the cultivable bacteria fraction, corroborated by axenic culture-based MALDI-TOF MS IDs. Particularly, functional analysis of $Rag2^{ko}$ mice gut microbiota proteins revealed the presence of abundant glutathione, riboflavin metabolism and pentose phosphate pathway components, possibly related to genetic background.

The metaproteomic pipeline herein presented may represent a useful tool to investigate the highly debated *onset* of the human gut microbiota in the first days of life, when the bacterial composition, despite its very low diversity (complexity), is still very far from an exhaustive description and other complex microbial consortia.

☆☆ This article is part of a Special Issue entitled: Trends in Microbial Proteomics.

* *Correspondence to:* A. Urbani, Department of Experimental Medicine, University of Rome "Tor Vergata", Via Montpellier 00133, Rome, Italy.

** *Correspondence to:* L. Putignani, Parasitology Unit, Metagenomics Unit, Department of Laboratories, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio 4, 00165, Rome, Italy. Tel.: +39 0668592598 2176; fax: +39 0668592218.
E-mail addresses: andrea.urbani@uniroma2.it (A. Urbani), lorenza.putignani@opbg.net (L. Putignani).

[1] Both authors are senior authors.

1874-3919/$ – see front matter
http://dx.doi.org/10.1016/j.jprot.2013.10.025

Biological significance
The manuscript deals with a "frontier" topic regarding the study of the gut microbiota and the application of a metaproteomic pipeline to unveil the complexity of this fascinating ecosystem at the very early stages of life. Indeed during these phases, its diversity is very low but the bacterial content is highly "instable", and the relative balance between mucosal and fecal bacteria starts its dynamics of "fight" to get homeostasis. However, in the neonatal period, especially immediately after birth, a comprehensive description of this microbial eco-organ is still lacking, while it should be mandatory to highlight its first mechanisms of homeostasis and perturbation, while it co-develops with and within the host species.
In order to unravel its low but almost unknown microbial community multiplicity, the newborn mouse gut, characterized by a "very" low complexity, was herein selected as model to design a LC–MS -based shotgun metaproteomic approach, potentially suitable to study onset and shaping[2]in human newborns. A microbiological semi-automatic computational analysis was performed to infer gut phylotypes; such as proof of evidence, related OTUs were compared to axenic-culture-based MALDI-TOF MS IDs showing consistency at family and phyla levels for the bacterial cultivable fraction.
This article is part of a Special Issue entitled: Trends in Microbial Proteomics.

1. Introduction

Complex ecosystems of host and microbial cells are organized into diverse ecological niches (*e.g.*, teeth, mouth, nose and respiratory district, gut, and genito-urinary tract) inside mammalian organisms. In each district different groups of microbes (phylotypes) form communities that change across space (*e.g.*, spatial variability determinants) and time (*e.g.*, age variability determinants) [1] Cohabitation with microbes is the product of a long evolutionary pathway resulting in a relationship based on mutual advantages that in humans is essential for health [2]. The human body together with "its" microbes has been defined as superorganism [3] and because hosting billions of microbes benefits their genes, proteins and metabolites acquiring additional functions and thus amplifying growth and survival tools. The superorganism description needs a new system biology-based approach, employing either metagenomic or metaproteomic strategies, which offers a new holistic paradigm of the human biological ontology [4].

The development of metagenomic studies has arisen from the need to interpret the vast data sets of sequences produced by the microbial genome projects [5], and to link their annotations to the functional counterpart in the context of body habitats. Indeed, in these habitats the bacteria establish a dynamic interplay with the host, generating the microbiome [6]. Metaproteomics, instead, analyzes protein patterns expressed in the ecosystems and thus describes the real-time dynamics of the systems [7]. In this context, a full comprehensive description of human gut phylotypes is essential to interpret gut homeostasis and perturbation [8–11].

In order to unravel gut microbial community multiplicity at the very early stages of life, the mouse gut, which is characterized by a lower gut complexity than the humans [12], may represent an appropriate model to design interpretative pipelines in metaproteomic analyses for studies on neonatal gut microbiota onset and development [13,14]. Indeed, during these stages, microbiota diversity is extremely low and the bacterial content is highly "instable", with a topographic and metabolic balance tremendously depending on the respective mucosal and fecal bacteria counterparts, which starts their fight dynamics to get homeostasis [15]. However, for obvious ethical issues studies on mucosal bacterial contents cannot be performed in neonatal age, and gut topographical-related results can be therefore inferred by employing newborn mouse models. Remarkably, the bacterial complexity and also its contents is particularly affected by the mucosal surface extension [16] hence providing low bacterial content which can actually be related to the very small surfaces of the newborn mice. Indeed, also the pivotal metaproteomic study of Li et al. [17] on the human mucosal luminal interface, performed on healthy adults enrolled in a cancer surveillance program, showed the limited presence of distinct proteins (*i.e.*, 49) associated to biogeographic features, suggesting a possible low diversity index of the mucosal bacterial-associated communities, which can be obviously more restrained in the very early life stages. In fact, to the best of our knowledge up to now, metaproteomic studies on mammalian gut microbiota have been mainly based on the fecal content, either gel-based [18–20], or gel-free [21,22] approaches.

However, sample complexity remains a very significant issue and the few metaproteomic studies performed on rodents gut microbiota have been exploiting either 1D-SDS gel electrophoresis or isoelectrofocusing as the first fractionation step, at the protein or peptide level, prior to LC–MS^2 injection analysis [12–14,23].

As a descriptive approach, we chose a common shotgun proteomics experiment, relying on a monodimensional reverse-phase (RP) chromatography, adjusting the gradient time in order to obtain the maximum spreading of peptides along the whole run time, thus minimizing ion suppression at the ESI source. Tandem MS was performed on a fast scanning 3D-ion trap, coupled to a Biotyper MALDI-TOF MS for the complementary analysis of the cultivable bacterial fraction. The herein study mainly aimed at describing mouse gut enterotypes during very early life stages (*e.g.*, programming phase), when limited microbial content is expected [15]