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Original article

Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus*

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ABSTRACT

An innovative metaomics approach integrating metatranscriptomics and metaproteomics was used to characterize bacterial communities in the microbiota of the Lyme borreliosis spirochete vector, *Ixodes ricinus* (Acari: Ixodidae). Whole internal tissues and salivary glands from unfed larvae and female ticks, respectively were used. Reused *I. ricinus* RNA-sequencing data for metranscriptomics analysis together with metaproteomics provided a better characterization of tick bacterial microbiota by increasing bacteria identification and support for identified bacteria with putative functional implications. The results showed the presence of symbiotic, commensal, soil, environmental, and pathogenic bacteria in the *I. ricinus* microbiota, including previously unrecognized commensal and soil microorganisms. The results of the metaomics approach may have implications in the characterization of putative mechanisms by which pathogen infection manipulates tick microbiota to facilitate infection. Metaomics approaches integrating different omics datasets would provide a better description of tick microbiota compositions, and insights into tick interactions with microbiota, pathogens and hosts.

1. Introduction

The microbiota plays an important role in several processes affecting human and animal health, agriculture, environment, and host-pathogen interactions (Kau et al., 2011; Schwabe and Jobin, 2013; Philippot et al., 2013; Bouchez et al., 2016). Next-generation sequencing or omics technologies can be used for microbiota characterization under different experimental and natural conditions. Metagenomics have been used to characterize the microbiota in different hosts including both model and nonmodel organisms such as humans and tick vectors (Clay et al., 2008; Andreotti et al., 2011; Carpi et al., 2011; Vayssier-Taussat et al., 2015; Qiu et al., 2014; Williams-Newkirk et al., 2014; Van Treuren et al., 2015; Narasimhan and Fikrig, 2015; Yoon et al., 2015; Abraham et al., 2017; Heintz-Buschart and Wilmes, 2017; Greay et al., 2018; Varela-Stokes et al., 2017; Xiang et al., 2017). Different metatranscriptomics approaches have been also applied to the study of microbial communities in arthropod vectors and vertebrate hosts (Mäder et al., 2011; Johansson et al., 2013; Vayssier-Taussat et al., 2013; Razzauti et al., 2015; Luo et al., 2017). Recently, metaproteomics and metabolomics have emerged as powerful tools for the characterization of dynamic host-microbiome interactions, particularly in combination with metagenomics and metatranscriptomics approaches (Tanca et al., 2013, 2014; Franzosa et al., 2015; Aguiar-Pulido et al., 2016; Cheng et al., 2017). Furthermore, metaomics or the integration of different omics approaches allows network-based analyses to describe the complexity and function of different biological processes involved in host/tick-pathogen and host/tick-microbiome interactions (Franzosa et al., 2015; Villar et al., 2015; Narasimhan and Fikrig, 2015), and the discovery of new targets for prevention and control of tick-borne diseases (Abraham et al., 2017; Narasimhan et al., 2017; Xiang et al., 2017).

Ixodes ricinus (Linnaeus 1758) (Acari: Ixodidae) are obligate hematophagous ectoparasites and vectors of multiple pathogens such as *Borrelia* spp. (Lyme borreliosis and hard tick-borne relapsing fever), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), tick-borne encephalitis virus (TBE), and *Babesia* spp. (babesiosis) (de la Fuente et al., 2008, 2017). Additionally, *I. ricinus* have a diverse community of commensal and symbiotic microorganisms which exert multiple effects on tick fitness, nutrition, development, reproduction, defense against environmental stress, immunity and transmission of tick-borne pathogens (Bonnet et al., 2017; de la Fuente et al., 2017). The *I. ricinus* microbiome was first characterized using a metagenomics approach (Carpi et al., 2011; Nakao et al., 2013; Bonnet et al., 2014). Vayssier-Taussat et al. (2013) characterized the bacterial community of

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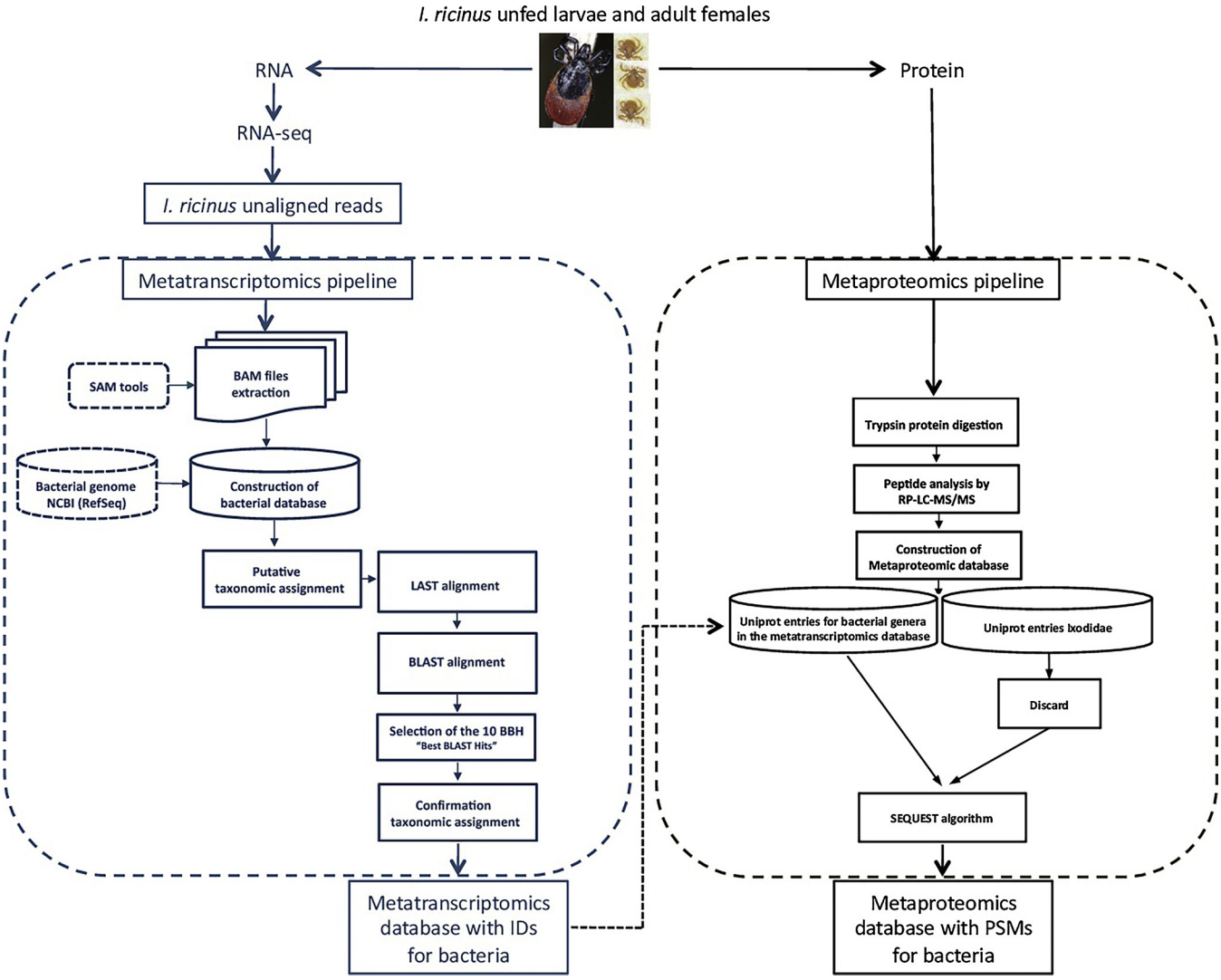

Fig. 1. Metaomics experimental design. An integrated metatranscriptomics and metaproteomics approach was developed for the characterization of *I. ricinus* bacterial microbiota. Reused *I. ricinus* RNA-seq data was the basis for metatranscriptomics analysis that resulted in the database of identified bacterial genera. This database was then use to generate the Uniprot protein database used in metaproteomics analysis.

I. ricinus using a whole transcriptomics approach, resulting in a better identification of previously unknown bacteria and accurate identification of potential pathogens. This method also provides a better understanding of the tick-microbiome interactions when compared to metagenomics. Additionally, reusing RNA sequencing (RNA-seq) data has been also used as an efficient strategy for the screening of pathogens in ticks (Zhuang et al., 2014a).

In this study, we used the integration of metatranscriptomics and metaproteomics for the characterization of the tick bacterial microbiota in unfed *I. ricinus*. Reused *I. ricinus* RNA-seq data for metranscriptomics analysis together with metaproteomics provided a better characterization of tick microbiome by increasing bacterial identification and support for identified bacteria with putative functional implications.

2. Materials and methods

2.1. Tick samples and processing

Tick samples were obtained and processed as previously described (Genomic Resources Development Consortium et al., 2014). Briefly, *I. ricinus* unfed larvae and adult females were obtained from the reference laboratory colony maintained at the tick rearing facility of the Institute of Parasitology of the Biology Centre of the Academy of Sciences of the Czech Republic. Whole internal tissues and salivary glands from 300 larvae and 30 female ticks, respectively were combined and used for RNA-seq. All ticks were washed with a series of solutions composed of tap water, 3% hydrogen peroxide, two washes of distilled water, 70% ethanol and two more washes with distilled water prior to dissection for DNA, RNA and protein extraction. Total DNA, RNA and proteins were extracted using Tri Reagent (Sigma-Aldrich, St. Louis, MO, USA) according to manufacturer instructions. RNA was further purified with the RNeasy MinElute Cleanup Kit (Qiagen, Valencia, CA, USA) and characterized using the Agilent 2100 Bioanalyzer (Santa Clara, CA, USA) in order to evaluate the quality and integrity of RNA preparations. DNA and RNA concentrations were determined using the Nanodrop ND-1000 (NanoDrop Technologies Wilmington, Delaware USA). Proteins were resuspended in 20 mM Tris-HCl pH 7.5 with 4% SDS and protein concentration was determined using the BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA) with bovine serum albumin (BSA) as standard.

2.2. Integrated metaomics experimental design

An integrated metatranscriptomics and metaproteomics approach was developed for the characterization of *I. ricinus* bacterial microbiota (Fig. 1). Reused *I. ricinus* RNA-seq data (Genomic Resources