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Application of high-throughput pyrosequencing in the analysis of microbiota of food commodities procured from small and large retail outlets in a U.S. metropolitan area – A pilot study

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ABSTRACT

With the advent of high-throughput sequencing technologies, it is possible to comprehensively analyze the microbial community of foods without culturing them in the laboratory. The estimation of all microbes inhabiting a food commodity (food microbiota) therefore may shed light on the microbial quality and safety of foods. In this study, we utilized high-throughput pyrosequencing of 16S rRNA genes as well as traditional microbiological methods to evaluate the bacterial diversity and the predicted metabolic pathways associated with the bacterial communities of selected foods (romaine lettuce, cabbage, deli meat, and chicken legs, total 200 samples) procured from small and large retail outlets located in Memphis-Shelby County, Tennessee, USA. For high-throughput sequencing, microbial genomic DNA was directly extracted from the food products and subjected to genetic sequencing. Aerobic plate count of all food samples was also performed. Foods from small stores (such as corner stores) were found to contain higher bacterial counts as compared to large stores (such as supermarkets). High-throughput pyrosequencing in tandem with bioinformatics analyses revealed a comprehensive picture of the bacterial ecology of foods at different taxonomic levels. *Firmicutes* and *Proteobacteria* were the most abundant phyla across all products. At the genus level, *Enterobacter* and *Pantoea* in vegetables, and *Bacillus* and *Aeromonas* in animal products were found to be the most abundant. The bacterial predicted metabolic pathways such as inosine-5′-phosphate biosynthesis I, methylglyoxal (MG) degradation pathways, urea cycle, dTDP-L-rhamnose biosynthesis I, and mevalonate pathway I differed in foods procured from small stores as compared to large groceries or supermarkets. The results from this study revealed that the bacterial ecology (both in terms of numbers and types of bacteria) of food commodities might differ based on the vending outlet type (large vs. small) of retail stores. The overall estimation bacterial communities in foods by high-throughput sequencing method may be useful to identify potential taxa responsible for food spoilage. Moreover, the data from pyrosequencing of 16S rRNA genes can also be applied to infer major metabolic pathways in bacteria inhabiting different foods. This may reflect the role of these pathways in food-bacteria interaction and adaptation.

1. Introduction

Broad spectrum identification of the entire bacterial community of food samples is possible with the advent of the next-generation sequencing (NGS) or high-throughput sequencing (HTS) of 16S rRNA gene (hereafter referred to as 16S rRNA community profiling) or shotgun metagenomics (Bhatt et al., 2012; Ercolini, 2013). The overall microbiota composition of a given sample can be deduced at different taxonomic levels by NGS without culturing the bacteria (Mukherjee et al., 2014; Mukherjee et al., 2016; Solieri, Dakal, & Giudici, 2013). In the field of food microbiology and food safety, the application of 16S rRNA community profiling or metagenomics has gained increasing

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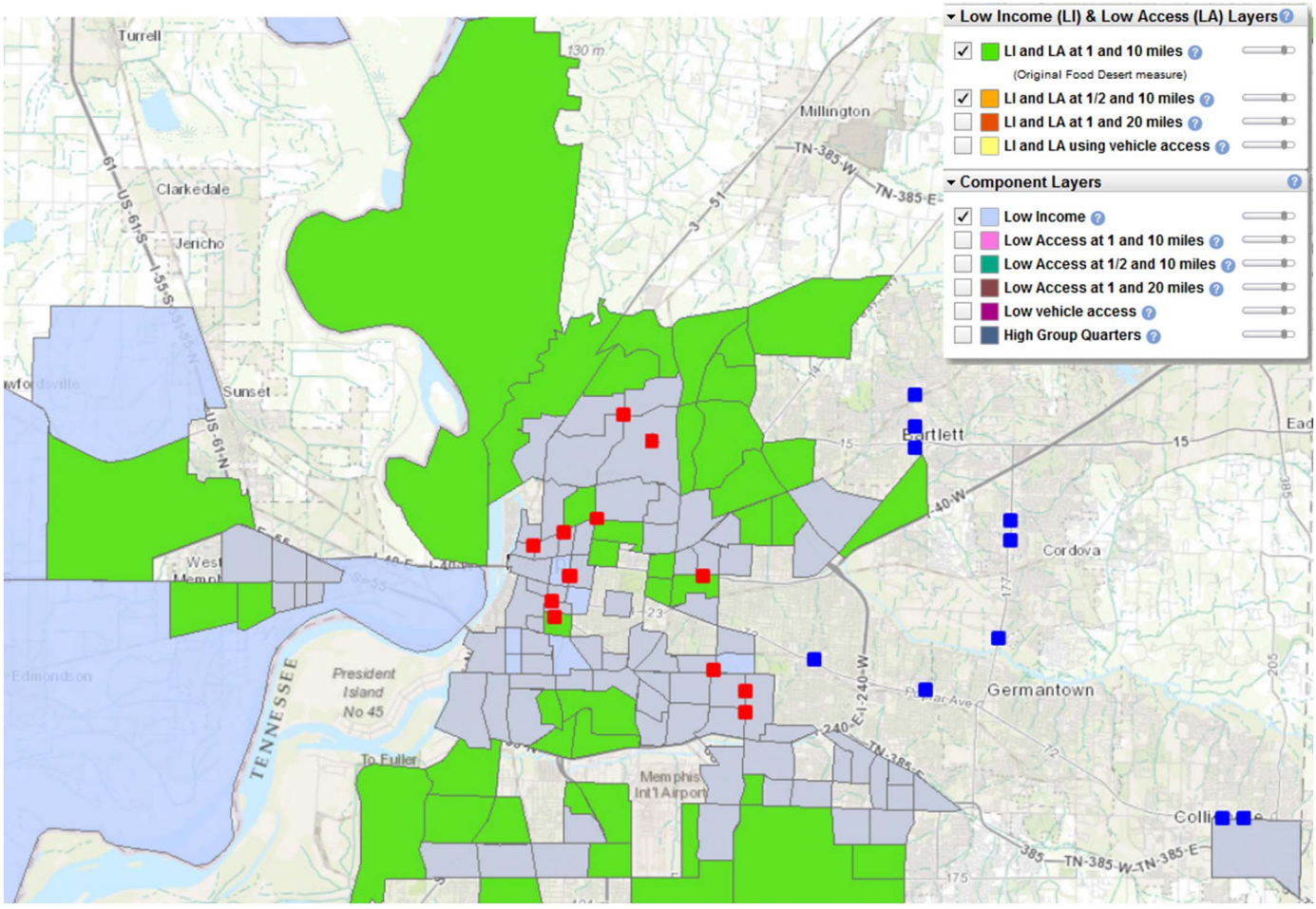

Fig. 1. Map showing the sampling area and sampling points. The stores in low SES areas are denoted by (■); while the high SES area stores are marked by (■). The background colors are indicative of food security measures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Data Source: Food Access Research Atlas, USDA-ERS (can be accessed at: http://www.ers.usda.gov/data-products/food-access-research-atlas/go-to-the-atlas.aspx [Accessed July 17, 2017].

attention in recent years, especially in studying the microbiota dynamics during food fermentation or food spoilage (Ercolini, 2013; Mayo et al., 2014). Some NGS studies also reported overall bacterial community diversity of different food commodities under different conditions (Grande Burgos, López Aguayo, Pérez Pulido, Galvez, & Lucas, 2017; Jackson, Randolph, Osborn, & Tyler, 2013; Xiao, Dong, Zhu, & Cui, 2013). Therefore, NGS methods can potentially be applied to understand the microbial community dynamics of foods in different points of food production chain, such as, during manufacturing, distribution, storage, and vending.

Foods sold at retail stores can be subjected to different environmental factors which may alter the composition of autochthonous microbes of foods, or introduce non-native microbes as contaminants, and may sponsor growth of pathogens. Environmental factors playing important roles in microbial composition, colonization, or even contamination in retail food stores may include (but not limited to) temperature of refrigeration, cleanliness of food contact surfaces (e.g., cutting boards, containers), employee hand-hygiene, packaging, cross-contamination, etc. Some of these factors are integral to food establishment inspection by local, state, or federal food safety inspectors. At a macro level, the outcomes of such inspections are interpreted as "food safety inspection scores". Often retail stores located in impoverished urban neighborhoods or low socioeconomic status (SES) areas score lower than major grocery stores and supermarkets, indicating substantial differences in retail food handling and vending environments depending on their point of sale (Pothukuchi, Mohamed, & Gebben, 2008). Large groceries or supermarkets usually allocate significant resources to comply with the best practices of food handling, storage, and vending. Also, most of the larger grocery-chains or supermarkets have robust employee training in place to ensure proper food handling and sanitary conditions. However, the situation for small food vendors and corner stores serving poor areas can be very different. Retailers who sell foods in food deserts/low income/poor/underserved neighborhoods (indicated as "low SES" throughout the manuscript) are often marginal businesses with limited resources, and face several challenges and barriers (such as, lack of business capital and infrastructure related to food storage and handling, intense price competition, high investment in insurance, higher employee turnover, and lack of sanitary awareness) that often force them to allocate fewer resources towards addressing critical issues (such as, compliances towards food handling guidelines) to ensure vending of food which is safe to consume (Pothukuchi et al., 2008). This differential food vending environments may result in altered microbiological composition of food commodities sold in retail stores located in different SES-locations, as reported by previous studies utilizing culture-dependent microbiological methods (Koro, Anandan, & Quinlan, 2010; Signs, Darcey, Carney, Evans, & Quinlan, 2011). These traditional microbial detection methods routinely utilized in food analyses are target microbe-specific and rely on isolation of target bacteria by culturing them on agar plates followed by detection using conventional microbiological methods (Doyle & Buchanan, 2012; Erkmen & Bozoglu, 2016), or by other biochemical and molecular methods such as enzyme-linked immunosorbent assay (ELISA) and