Metagenomic Analysis of Bacterial Communities in Food Spoilage

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Abstract

Food spoilage and food-borne illnesses are threats to global health and economy. Some food-borne illnesses have long-standing effects. The current global estimate of 600 million cases of food-borne illnesses per year is underrepresented because of underreporting. Children under the age of 5 years are highly vulnerable to food-borne diseases and they remain the major carriers. Food-borne illnesses arise from food spoilage and can be caused by chemicals and/or different types of microorganisms such as bacteria, viruses, fungi, parasites, etc, wherein food spoilage caused by bacterial communities is more common. Both spore-forming and non-spore-forming bacteria can cause food-borne illnesses. Current techniques for assessing food safety have several limitations. Culture techniques and polymerase chain reactions are labour intensive, require a long time to obtain conclusive results, associated with considerable bias, and hence not suitable for addressing rapid outbreaks [*1*]. The advent of metagenomics enables direct analysis of genomes and is capable of revolutionizing food safety analysis. The technique grants access to the genetic composition of microbes, especially the unculturable ones. Apart from the diagnosis of food-borne illnesses, metagenomic sequencing and library generation enable food safety monitoring, and better preparation of the population for future outbreaks. Whole genome sequencing remains the backbone of metagenomics and there has been an explosion in the metagenome shotgun curated datasets in the past few years. The advantages are numerous when compared to either 16S rRNA gene fingerprinting or error-prone epidemiological strategies which are significant for the fast and reliable identification of the source of microbial infection.

Key Words: food borne diseases, Metagenomics, Metagenome shotgun, 16S rRNA.

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1. Introduction

Food is essential for life, and it is extremely important to ensure safe food and water to the population. Food-borne illnesses pose a global threat to health and the economy, and hence novel diagnostic methods to ensure food safety are essential[*2*]. The world population is skyrocketing and expected to reach 9 billion by 2050[*3*]. Each year contaminated food causes enormous morbidity and mortality[*4*]. The latest reports indicate that food-borne pathogens can cause illnesses in 600 million people and 420000 deaths[*5*]. There are four major challenges of food safety such as microbiological safety, chemical safety, personal hygiene, and environmental hygiene. The impact of food-borne illnesses on the economy is several folds higher in low- and middle-income countries[*6*]. Compared to adults the diseases spread fast among children and they are the major carriers. Currently microbial and chemical contaminants are the major causes of mortality among children[*7*]. Food-borne associated deaths are more in underdeveloped regions compared to developed countries[*8*].

1.1 Importance of food safety

Food safety deals with the scientific aspects by which food (raw or cooked) is handled, prepared, and stored so that there are no food-associated illnesses. Along with sustainable methods of production of food, it is also important to devise strategies for food safety on a global scale[*9*]. Urbanization and globalization had their impacts on food safety, and it is notable that food safety, food security, and nutrition are interlinked[*10*]. Although advances in novel scientific techniques played a major role in the rapid assessment of causative agents for food-borne illnesses globalization had both positive and negative effects on food safety[*11*].. Apart from causing illnesses, food spoilage can also affect global food availability.

1.2 Bacterial food spoilage and its outcome

Bacteria, viruses, fungi, and parasites can cause food spoilage[*12*]. However, the predominant non-spore-forming ones are bacteria such as those belonging to *Lactobacillus spp.*, *Carnobacterium spp.*, *Leuconostoc spp.*, *Pediococcus spp.*, *Lactococcus spp.*, *Weisella spp.*, *Streptococcus spp.*, and *Kurthia zopfii*, and *Brochothrix thermosphacta* [*13–19*]. These bacteria can cause changes in flavour, colour, odour, pH and produce gas and slime. The spore-forming bacteria include those belonging to the aerobic *Bacillus* species and the

anaerobic *Clostridium* species[*20*]. *Alicyclobacillus*, a thermophilic acidophilic spore-forming bacteria can spoil acidic beverages[*21*]. *Desulfotomaculum nigrificans* is also another thermophilic bacterium reported to cause food spoilage. The consumption of food contaminated by one or more of the bacteria mentioned above can cause irritations in the gastrointestinal tract as implicated by intestinal cramps, vomiting, nausea, fever, diarrhoea, etc indicating gastroenteritis[*22*]. There are pathogenic spore-forming bacteria responsible for food-borne illness outbreaks such as those belonging to *Brucella spp*., *Campylobacter spp*., *Salmonella spp*., *Yersinia spp*., *Listeria spp*., *Escherichia coli spp*[*23*].

1.3 Importance of metagenomics in the identification of bacteria

The advances in metagenomics have considerably revolutionized diagnosis of bacterial food spoilage. Metagenomics is indeed a next-generation disruptive technology that may be used to ensure food safety[*24*]. The assays and data analysis of metagenomics enables the identification of complete microbiomes of food, its ingredients, and environmental samples. It also helps in quantifying the microbial population[*25*]. Since there is no need for culturing the sample, rapid diagnosis is possible considerably reducing the time and bias. Most importantly metagenomic approach can rapidly diagnose emerging risks that are previously unknown. Routine application of metagenomics can also indicate variations in the type and number of microbes present in the food and alert the concerned experts which allows swift action for investigation and potential remediation[*26*].

2. Identification of bacterial communities in food using metagenomics

Metagenomics can be effectively used for bacterial strain profiling[*27*]. This has become possible because of the advances in whole genome sequencing (WGS) and computational techniques[*28*]. Since the sample preparation and extraction of microbial DNA can considerably affect the accuracy and quality of results, utmost care must be taken during sample collection and preservation, with measures to minimize sample contamination. The sample size can be determined by pilot experiments since the microbial population can be diverse in different samples, although collected from the same environment[*29*]. Other factors which influence the results are freeze-thaw cycles, duration of sampling, and storage conditions[*30*]. The problem associated with the low number of pathogenic microbes can be overcome to a certain extent by immunomagnetic separation, short enrichment, multiple-displacement amplification etc., used for simultaneous identification of *E. coli* and *Salmonella*[*31–33*].

2.1 Microbial DNA extraction and Host depletion

Microbial DNA extraction should focus on maintaining the quality and quantity of DNA. The challenge is to isolate DNA when the microbial level is very less, and the chances of contamination from handling and reagents are high. Both mechanical and chemical techniques may be used for DNA extraction. Bead beating and sonication are mechanical disruption methods that are used in the case of lysis-resistant gram-positive bacteria and fungi. However, bead beating which uses small silica and zirconium beads can cause DNA shearing resulting in shorter DNA fragments and hence negatively affect fragment size selection and library preparation. In addition to using high-quality reagents, it is necessary to employ controls with no template. Since host DNA is the major source of contamination, host DNA must be depleted before analysis to increase sensitivity[*34*]. Saponin treatment, osmotic lysis using sterile water and using a host-specific DNase can be used for host-DNA depletion. There are several commercial kits for rapid and effective host DNA depletion[*35*].

2.2 Differentiation of dead and live bacteria

One significant challenge in the metagenomics approach is the differentiation of DNA between dead and live bacteria. Although several techniques such as the use of DNA intercalating chemicals namely propidium monoazide before 16S rRNA can increase the concentration of DNA from viable cells, studies indicate that there can be considerable impact on the sensitivity, especially when there is significant complexity of microbial flora[*36*]. Shotgun metagenomics is a highly discussed technique that enables the evaluation of the diversity and abundance of bacteria in a complex environment. The technique is useful in studying unculturable microbes. Most importantly it can read all the genomic DNA unlike 16S rRNA sequencing and can be used for the simultaneous identification and profiling of various microbes[*37*].

2.3 Sequencing and library preparation

Metagenomic sequencing enables the identification of the microbial diversity of a sample and the taxa present. Once the DNA sample is isolated and purified, it is quantified. The sequencing and library preparation should employ a control sample, especially when using a new type of sample. The control samples may be mock samples or even well-characterized samples from sample banks. The targeted library preparation in metagenomic sequencing strategies such as metabarcoding starts with the selection of a single target which is an informative region in the genome to identify the species, followed by amplification and sequencing of this target. 16S rRNA sequencing has been a mainstay of bacterial analysis for several decades which allows

the identification of bacterial phylogeny and taxonomy and allows sequencing of the highly conserved 16S rRNA gene[*38*].

3. Types of metagenomic techniques and their uses

The sequencing and library preparation can use different approaches such as Metabarcoding sequencing, Shotgun metagenomic sequencing, and Long-read metagenomic sequencing. Each technique has its advantages and limitations.

3.1 Metabarcoding

Metabarcoding enables the identification of a highly diverse microbial community[*39*]. Bacterial 16S rRNA can be used as a target, and reconstruction of the complete genome is not required. Compared to other techniques there are several tools and curated databases which allow easy classifications based on taxonomy. The technique also allows the comparison of changes in abundance with respect to different samples and treatments. When there is less complexity the technique also allows the identification of rare taxa. Metabarcoding is cost-effective and can be conducted using most of the platforms and sequencers currently available[*40*]. However, it has several limitations. Most importantly the targeted sequencing approach requires prior information on the target microbial group. Universal targets such as 16S rRNA may not be suitable since it has variability in abundance across different taxa, and hence it is not possible to identify the pathogenic strains, the subtypes, or the viability of bacteria. The technique is also not suitable to identify novel microbes, and determine the absolute abundance. Moreover, significant expertise and computational tools may be required[*41*].

3.2 Shotgun sequencing

In the shotgun sequencing approach, all genomic DNA is sequenced and is suitable for less diverse microbial communities. Compared to metabarcoding sequencing, the amplification bias is less. It is possible to identify the targets. The technique is also suitable for identifying novel subtypes of microbes and can be performed on the currently available platforms and sequencers. However, since the technique does not use any specific target, only dominant genomes are represented well and are less sensitive compared to metabarcoding. It is also not suitable for differentiating live bacteria from dead ones. Depending on the depth of sequencing,

type of sample, and microbial population to be identified, the technique can be costly and requires moderate to high expertise[*42*].

3.3 Long-read metagenomics

Genomic DNA or RNA can be sequenced using a long-read metagenomics sequencing platform, and is suitable for less diverse microbial communities, with a few dominant species[*43*]. Compared to metabarcoding, the amplification bias is less, and enables identification based on the phylogenetic markers[*44*]. It is possible to identify the modified bases using this sequencing approach and is possible to identify novel types of bacteria[*45*]. It is also possible to perform the study in the field itself[*46*]. However, since the base callers are trained on a specific data set, the technique requires pathogen-specific base callers[*47*]. It is less expensive than shotgun sequencing and costlier than metabarcoding. The technique cannot differentiate between live and dead microbes. There are limitations in the availability of computational tools for analysis and requires expert supervision.

5. Implementation of metagenomics approaches to the food industry: advantages and disadvantages

In the food industry, proper implementation of culture-independent diagnostic tests such as metagenomics approaches can be used for the discovery of new pathogens which enables better management of foodborne illnesses[*48*]. Accurate characterization and identification of pathogens is possible by omics approaches[*49*]. However, there are potential hurdles associated with the identification of newly described microbes such as matching sequences based on whole genome sequence data. However, the bioinformatics tools are costly and hence metagenomic approaches are expensive compared to conventional assays, although multiple pathogenic microbes may be detected rapidly in a single test which may manage the cost[*50*]. One limitation of the metagenomic approach is the lack of bioinformatics expertise and regulations, although interpretation of the results is possible to a certain extent by automated data analysis. Currently, there is a lack of standardized methods for the reliability and comparability of results. Since there are no available reference materials, sample preparation, sequencing methodology, and analysis must be given attention, and there is a requirement for representative data sets and databases exclusively for food safety. After the advent of

metagenomics the data is open to all, unlike conventional techniques. This allows sharing the data which may further improve global food safety and security. Apart from the detection of a pathogenic microbe, the metagenomics approach may provide an early indication of food safety and quality which can prevent possible food-borne disease outbreaks. However, the current data storage cost may not be affordable and considerable regulations should be in place regarding the ownership, liability, and security of data. The most important limitation of the metagenomics approach compared to the conventional technique is the inability to distinguish the live organisms from the dead ones which is significant while monitoring an intervention. Metagenomics approaches may eliminate costs associated with the long-term storage of isolate and negates the requirement of biobank reference isolates and reanalysis during development of future technologies. However, still there are no proper regulatory frameworks available and moreover regulators may have to be trained for the new approach.

6. Conclusion

Metagenomics is a disruptive tool that grants access to the enormous diversity of the microbial world, especially the unculturable microbes. It is a powerful tool for identifying the pathogens associated with food-borne illnesses, apart from its applications in identifying novel enzymes and bioactive molecules from microbes for potential applications in the microbiology, food, and pharmaceutical industry. Metagenomics is backed by the rapid establishment of WGS as a crucial tool for the surveillance of food safety. It plays a key role in monitoring the microbial communities to prevent the spread of food-associated microbial hazards thereby establishing food security. The current advancements indicate that a universal test kit for pathogen detection is not far from reality. Apart from establishing food security metagenomics also offers quality control and screening of unauthorized genetically modified foods. However, on a global scale, there is a significant requirement for training, recruitment, investment for infrastructure, partnership with regulatory organizations, and the establishment of both national and international guidelines, for better application of metagenomics for food safety. It is also possible to educate highly susceptible individuals such as children, pregnant women, and immunocompromised individuals regarding safe food handling and proper hygiene practices to reduce the outbreaks of food-borne illnesses.

Conflict of Interest

The authors have no conflicts of interest to declare.

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