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## DNA metabarcoding of microbial communities for healthcare

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High-throughput sequencing allows obtaining DNA barcodes of multiple species of microorganisms from a single environmental sample. Next Generation Sequencing (NGS)-based profiling provides new opportunities to evaluate the human health effect of microbial community members affiliated to probiotics. DNA metabarcoding may serve as a quality control of microbial communities, comprising complex probiotics and other fermented foods. A detailed inventory of complex communities is a pre-requisite of understanding their functionality as whole entities that makes it possible to design more effective bio-products by precise replacement of one community member by others. The present paper illustrates how the NGS-based DNA metabarcoding allows profiling of both wild and hybrid multi-microbial communities with the example of a kombucha probiotic beverage fermented by yeast-bacterial partners.

**Keywords:** DNA metabarcoding, microbial communities, healthcare, probiotics

### Introduction

#### *High-throughput sequencing allows obtaining DNA barcodes of microbial communities*

DNA barcode is a short DNA sequence, selected in the genome, that can be used to identify different species, analogously to the black stripes of the Universal Product Code used to distinguish commercial products [1]. The traditional barcoding is designed to identify species isolated from individual specimens using Sanger DNA sequencing. For different groups of organisms the specific barcodes were proposed. For example, the Fungal Working Group has identified a ribosomal internal transcribed spacer (ITS) as the best barcode region for fungi [2, 3]. The gene of 16S RNA became a universal barcode for prokaryotic microorganisms [4]. The standardized barcoding is limited in the identification of only those species, which can be

cultured in laboratory conditions that made species profiling incomplete. The introduction of next generation sequencing (NGS) advanced significantly the applicability of the barcoding approach by making it faster, cheaper and capable of simultaneous identifying species of different taxonomic groups, including those, which cannot be cultured [5]. The DNA metabarcoding is referred to the automated identification of multiple species from a single bulk sample, containing entire organisms, or from a single environmental sample, containing degraded DNA [6]. Compared to the standard approaches, metabarcoding by pyrosequencing provides taxonomically more comprehensive data and allows faster performing of large-scale biodiversity research and reducing the labor consumption during sample processing as well as the overall cost of analyzing the bulk samples [7, 8]. The DNA metabarcoding approach has a wide range of applications in forensics, ecology and paleoecology.

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gy. In this review, we highlight the use of metabarcoding for a quality control of beverages and fermented products and for designing new probiotic beverages.

### *Functional beverages and analysis of microbial communities in fermented products*

DNA metabarcoding of microbial communities as an instrument of community analysis is of great importance for resolving many healthcare problems. The U.S. National Institute of Health has initiated the Human Microbiome Project to characterize the human microbiota and actually to design the personal DNA metabarcode of microbial communities (bacterial, archaeal and fungal) of humans. Metabarcoding of the human gut microbiota showed that different people harbor remarkably dissimilar microbiota, which may be affected by external factors such as lifestyle and dietary specificities [9]. Long-term studies in the United States demonstrated a nearly 100 percent reduction in the rate of coronary heart disease, diabetes in women and colon cancer in men in patients accepting “healthy lifestyle” (reviewed in [10]). The conception of healthy lifestyle assumes consuming the fermented beverages as natural probiotics, as a part of the healthy diet, e.g., yogurt, miso, tamari and kombucha. The food industry needs to assess carefully the safety and efficacy of all new species and strains of probiotics before incorporating them into the food products. The nutraceutical fermented products are produced by complex microbial communities characterized by rather unstable species composition. Using the DNA metabarcoded pyrosequencing approach, the profiling of complex yeast-bacterial/archaeal communities in diverse beverage fermentations allows the control of fermentation processes.

*Probiotics* are viable microorganisms, the health effect of which is independent of the site of action and the route of administration [11]. Living probiotic microorganisms occupy a wide niche because of keeping gut health [12], boosting immune system [13], memory and mental sharpness [14], preventing cancer [15], allergic disorders [16], diabetes and diabetes-induced impairments [17], etc. *Prebiotics*, in

contrast to probiotics, are non-digestible food ingredients like oligosaccharides, which fuel beneficial host-associated microbiota and enhance the positive action of probiotic microbial organisms. Health-promoting products, containing a combination of prebiotic and probiotic, are termed *synbiotics* [11]. One-third of cancer cases might be prevented by the nutritious healthy diet, which also maintains healthy weight and physical activity by consuming synbiotics. Further, the significance of tailor-made prebiotics, probiotics and synbiotics in cancer prophylaxis due to the bio-antimutagenic and desmutagenic activities has been reported [15]. Because of the lactose maldigestion problem and allergy to milk proteins, which are widespread in human population, the fermented non-dairy products seem to become more preferable [18]. These products also are cholesterol free and rich in neuroactive amines tyramine and histamine, as well as in antioxidants, which protect consumers from free radicals.

*Fermented probiotic products are produced by complex microbial communities* and characterized by rather unstable species composition, which strongly depends on the nutritional sources and possible contaminations. Usually, there exists a core consortium of microorganisms, which establish mutualistic relationships and get rid of any contaminating intruders due to a successful competition and the production of antimicrobial metabolites. On the other hand, the accessory members of the brewing communities are more important for the quality of the final product, and the need in a robust control of these communities is widely recognized. Taking into account that the majority of microbial species are uncultivable and cannot be detected by any microbiological methods, the DNA metabarcoding remains the only known tool to examine and control the fermented food microbial communities. This approach is suitable for the analyses of archaeal, bacterial and yeast diversity in natural fermented foods [19–25] and it is of great importance for a new probiotic/synbiotic design.

In this paper, we illustrate how this approach aims at the analysis of hybrid complex microbial commu-

nities using as example the DNA metabarcoding of a modified kombucha microbial consortium.

### *The DNA metabarcoding of a modified kombucha microbial consortium*

Kombucha beverage is becoming increasingly popular around the world today as a prophylactic probiotic and healing agent [26], and both the kombucha brewed drink and processed pellicles may be promising probiotic/synbiotic formulations [27]. The presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, minerals in the brewed drink and cellulose pellicle was reported [26]. The prophylactic and healing effects have been proven in a wide range of laboratories [28–32]. For example, kombucha tea can be considered as a potential strong candidate for future application as a functional supplement for the treatment and prevention of diabetes [32]. Kombucha culture is an example of a multi-microbial community with strong mutualistic relationships between its members – bacteria and yeasts. The studies on cultures have revealed that Kombucha Microbial Community (KMC) may include a variety of yeast species and acetobacteria (reviewed in [27]). During the fermentation of sugar in the tea extract the community members produce a big variety of organic molecules, which prevent KMC from colonization by other microorganisms. Kombucha culture is promising for a creation of consortia with desired probiotic properties. One of such approaches is based on constructing the hybrid ecotypes of KMC enriched by concomitant microorganisms originated from other fermented products, which are compatible with KMC. Any hybrid ecotype of KMC has been analysed by the DNA barcoded pyrosequencing of the whole hybrid kombucha community DNA and compared to the parental kombucha community structure.

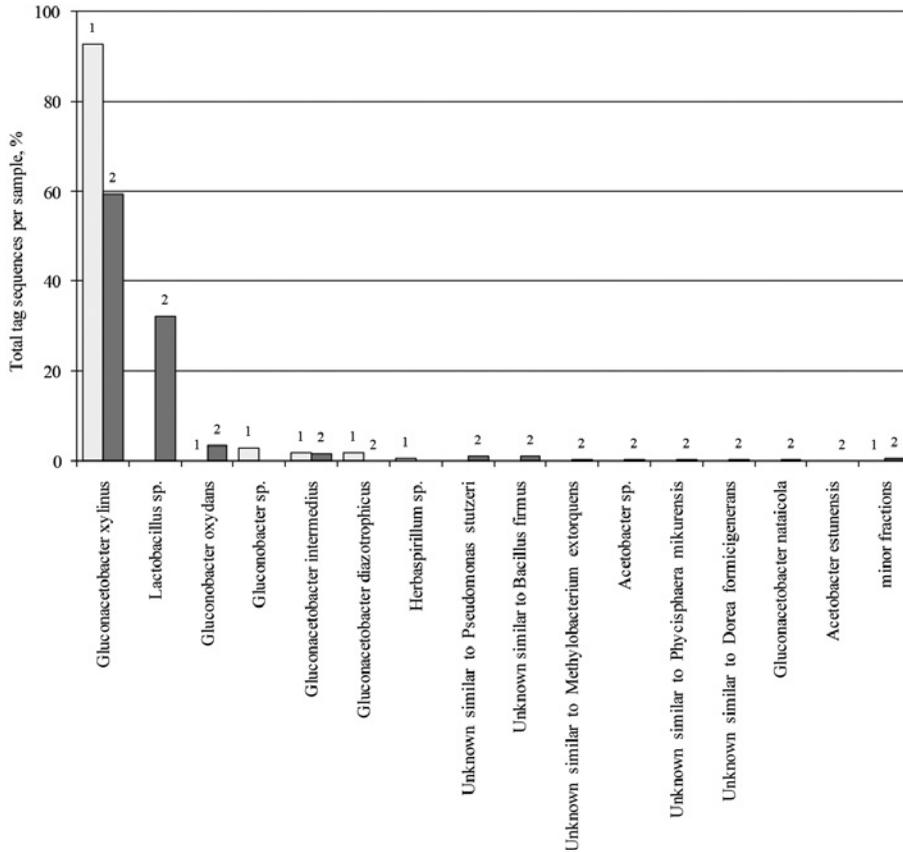
In advance, a non-sterile cabbage juice was inoculated by a kombucha culture and incubated for fermentation. The pellicle formed on a top of cabbage brine was transferred from the resulted fermented product to a fresh portion of medium for kombucha culturing (sweet black tea) and sub-cul-

tured several times. Total DNA has been extracted from cultural liquid and pellicle. The DNA metabarcoding has been done by using amplification of 16S rDNA and ITS regions and further pyrosequencing for identification of bacterial and yeast species, respectively [33]. The resulted metagenomic data, which summarized the most frequent BLASTN hits, are shown in the histogram in Figure.

In summary, the results of pyrosequencing showed that the hybrid kombucha culture is enriched by bacterial and yeast operational taxonomic units, which were not present in the stock KMC [33]. For example, *Lactobacillus* sp. has been revealed in hybrid KMC bacteriome, which was not reported before in the stock KMC. However, lactobacilli are typical in other kombucha ecotypes [25, 34] and fermented cabbage products [21] (see Figure). The brewing communities may be important for the quality of the final product, and the ability to introduce new members into KMC is of practical importance as a possible way to improve the medicinal properties of the kombucha product. For example, *Lactobacillus* sp. isolated from the hybrid kombucha could be introduced into a newly constructed kombucha communities with the defined core bacterial and fungal species more readily than some other lactobacilli strains selected from culture collections. Yeast DNA barcoding discovered a much higher number of OTUs in both pellicle and cultural liquid in the hybrid kombucha culture as compared to the parental KMC [33]. Many OTUs were not affiliated to any taxonomic unit because of too weak sequence similarity or showed similarity to unknown and uncultured microorganisms. Represented data show that the promising step in manufacturing of safe probiotics and synbiotics is to design synthetic mini-kombucha communities based on concomitant beneficial and harmless microbial species, using DNA barcoded microbial community profiling.

### **Conclusion and future perspective**

DNA metabarcoding of microbial communities may serve for many purposes, including quality control of beverages and fermented products. Analysis of



**Figure.** Relative normalized abundance of the most frequent operational taxonomic units of the hybrid kombucha microbial culture identified by top scored BLASTN hits by 16S rDNA reads in the cellulose-based biofilm in comparing with OTUs binned in DNA reads from parental kombucha culture film grown in a sterile black tea with sucrose (sBTS). Numbers of identified reads were normalized by the total numbers of reads in the samples.

DNA metabarcoding data allow mining more complete information about the structure and dynamics of changes in the microbial communities under specific conditions. These data can be used for the prediction of intrinsic relationships in complex microbial communities comprising bacterial, archaeal and fungal taxa and the estimation of the species diversity of natural populations. The DNA metabarcoding displays hidden microbes in microbial communities, which may be of great importance for this community and the probiotic manufacture.

The DNA metabarcoding by pyrosequencing is a new powerful tool for characterizing the dynamic changes in the probiotic communities and in the gut microbiota treated with probiotics. This knowledge may aid in improving the probiotic administration for prophylaxis of human diseases. The profiling of individual human microbiomes may be of great importance for the prognosis of diseases predisposition, su-

pervising the course of diseases and development of an individual therapy based on the probiotic administration. The NGS-based species profiling provides new opportunities to evaluate the effect of both individual and community members of probiotics in relation to individual human health. This molecular tool will host local clinics in the future as a routine method aiming at the improvement of health prophylaxis.

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#### **ДНК-метабаркодинг мікробних угруповань для підтримки здоров'я**

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Високопродуктивне секвенування дозволяє отримати штрих-коди ДНК декількох видів мікроорганізмів з однієї проби навколишнього середовища. Профілювання видів на основі технологій секвенування нового покоління (СНП) дає нові можливості для оцінки впливу членів мікробних угруповань пробіотиків на здоров'я людини. **Метабаркодинг** ДНК може слугувати для контролю якості мікробних угруповань, включаючи складні пробіотики та інші ферментовані продукти. Детальна

інвентаризація складних угруповань є передумовою розуміння їх функціональності як цілісного утворення, що дасть можливість створювати більш ефективні біо-продукти шляхом точної заміни одного з членів угруповання/спільноти іншими. Ця стаття показує, як можна застосувати метабаркодинг ДНК на основі СНП для профілювання диких і гібридних мульти-мікробних угруповань на прикладі пробіотичного напою комбучі, ферментованого дріжджово-бактеріальними партнерами.

**Ключові слова:** метабаркодинг ДНК, мікробні угруповання, охорона здоров'я, пробіотики

#### **ДНК-метабаркодинг мікробних сообществ для поддержания здоровья**

И. Е. Заец, О. В. Подоліч, О. Н. Рева, Н. А. Козыровская

Високопродуктивное секвенирование позволяет получить штрих-коды ДНК нескольких видов микроорганизмов из одной пробы окружающей среды. Профилирование видов на основе технологий секвенирования нового поколения (СНП) дает новые возможности для оценки влияния микробных членов сообществ пробиотиков на здоровье человека. **Метабаркодинг** ДНК может служить для контроля качества микробных сообществ, включая сложные пробиотики и другие ферментированные продукты. Детальная инвентаризация сложных сообществ является предпосылкой понимания их функциональности как целостного образования, давая возможность создавать более эффективные био-продукты с помощью точной замены одного из членов сообщества другими. Эта статья показывает, как можно использовать метабаркодинг ДНК на основе СНП для профилирования диких и гибридных мульти-микробных сообществ на примере пробиотического напитка комбучи, ферментированного дрожжево-бактериальными партнерами.

**Ключевые слова:** метабаркодинг ДНК, микробные сообщества, охрана здоровья, пробиотики

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