Exploration of the Human Oral Microbiome Using Next Generation Sequencing

– A Great Time to Be a Microbiologist!

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Humans can be considered "superorganisms" because they harbor microorganisms (microbiota) that contribute to normal human physiology. In fact, the vast majority of cells in the human body are not human at all – rather, they are microbial (~90 percent) (1). Most of these microbes are beneficial to human health. However, some microbes may also predispose humans to various diseases. Disruptions to the "normal "microbiota have been linked to a variety of diseases, such as inflammatory bowel disease and Crohn's disease of the colon. Similarly, some microbes in the oral cavity are involved in acute coronary syndrome, premature stillbirths, and a disease that produces destruction of the tissue of the mouth and cheek (cancrum oris, Figure 1). The dichotomy between "normal" and "not normal" microorganisms has lead some researchers to consider the microbial communities as major contributors to the healthy or the diseased states (2). Yet, what constitutes "normal" and "not normal" microbial communities is not well defined, especially in the case of microbes that live in the human oral cavity.

Until recently, microbiologists believed that 700 different microbial species colonize the human oral cavity (3), and that the microbes organize themselves in complex, multispecies associations attached to our tissues. These associations are commonly referred to as biofilms, which are aggregates of cells encased in extracellular polysaccharide matrices. Biofilms have different complexities, depending upon their locations, microbial composition and environmental conditions. The microbial composition of oral biofilms is different among individuals and can even vary within the different locations in the oral cavity of one individual. But new research based on Next Generation Sequencing (NGS) suggests that the composition and diversity of microbes in the human oral cavity are far more numerous and complex than previously believed.

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NGS has revolutionized the way scientists explore and understand the biological world. Since the commercial introduction of NGS technology, there has been an explosion of studies that have used this technique to sequence the whole genomes of different organisms, and even of communities of organisms. The projected number of peer-reviewed published papers dealing with NGS is expected

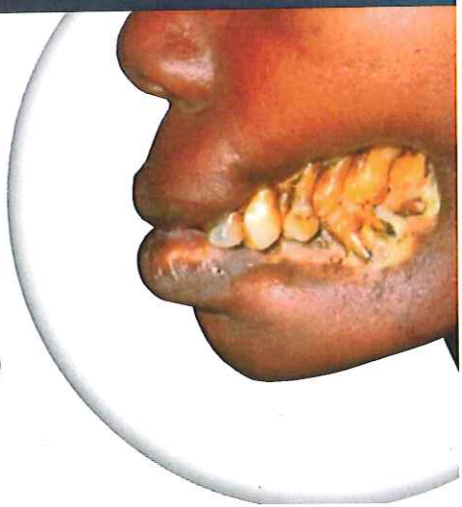

Figure 1. *Cancrum oris* occurs in people who live in extreme poverty. It can occur at any age and is often associated with people who have a weakened immune system due to poor nutrition and living conditions. Note the erosion of soft tissues, which is due to a mixed microbial community that attacks the dental and bony tissues (image from http://nf.churchinsight.com/Images/content/150/142707.jpg)

to reach 876 articles in 2010 and surpass 2,655 articles in 2011 (Figure 2, left panel) (based on records obtained from the Web of Science). Thus, we are witnessing a major paradigm shift in the exploration of biology that students and faculty at Alabama State University (ASU) are trying to capitalize on.

Why is NGS better than conventional sequencing?

Conventional chain termination sequencing involves the use of short DNA segments (primers) and the sequential incorporation of labeled and modified nucleotides onto a single DNA template (4). NGS is quite different from chain termination sequencing, because it does not require knowledge of the DNA template to be sequenced (i.e., no primer design). NGS is based on a massively parallel process involving the sequencing of hundreds of thousands of templates all at the same time (5,6). On a practical level, NGS is very straightforward: one collects a biological sample, extracts the DNA, processes the DNA following the manufacturer's protocols, and then deposits the processed DNA into the NGS device. In less than 10 hours, the sequence data is automatically output to a database. After two hours of data processing, the identities of the DNA sequences in the sample are determined by comparing the sequences to reference genes existing in DNA databases. The clear advantage of NGS over conventional sequencing is that hundreds of thousands of DNA templates can be sequenced in a single run. For example, a new model of a pyrosequencer, which was scheduled for release in April 2010, allows one scientist to sequence a small bacterial genome (e.g., 160 Kbp) in a week for less than $10,000. Fifteen years ago, the same genome would have taken several months, involved multiple people and cost at least $80,000. Using the genome of Haemophilus influenza as an example, which has 1.8 Mbp, the cost of sequencing back then was 50 cents per base.

What does NGS tell us about the diversity and composition of microbes in the oral cavities of healthy people?

Recent NGS-based studies have revealed that instead of 700 different microbial species inhabiting the oral cavity, there are about 25,000 different species (7). With respect to distinguishing between "normal" versus "not normal" microbial communities in the human oral cavity, we are halfway there. A recent study supporting the concept of a healthy "core" microbiome (i.e., the totality of microbes: their genetic elements and environmental interactions in a defined environment) in the human oral cavity has just been published (8). Yet, we still do not know if an unhealthy "core" microbiome exists in humans. Looking at Figure 1, we are left to wonder if a core microbiome is responsible for cancrum oris. If so, what are the successional events leading to the establishment of this core, and how can we prevent cancrum oris from occurring? Clearly, longitudinal clinical trials that sequence oral samples from individuals at healthy and disease stages are needed to address these questions. Here, the utilization of NGS technology is key for determining if core microbiomes exist in the oral cavity of healthy and compromised individuals, and for investigating the response of microbial communities to different treatment regimes.

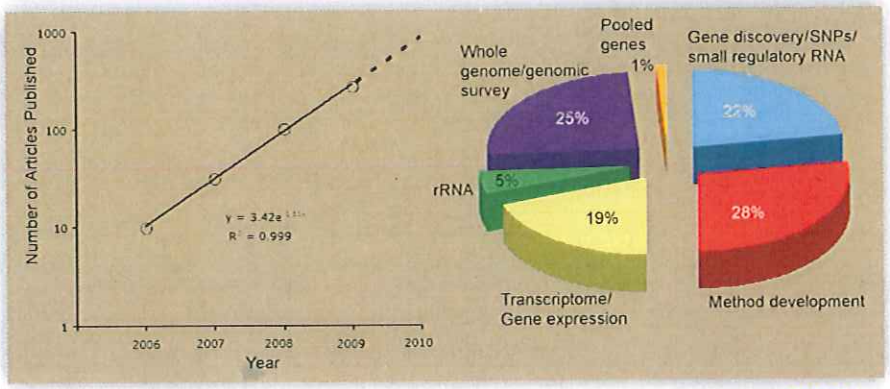

Figure 2. Cumulative number of articles published in the Web of Science dealing with Next Generation Sequencing (left panel) and a breakdown on their primary content (n=265 articles) (right panel). The analysis was based on the following search words: [454 pyrosequencing] OR [454 sequencing] OR [Illumina Solexa sequencing]. Based on the linear regression (shown in left panel), it was predicted that 875 articles would be published in 2010 and 2,655 articles in 2011.

What else can NGS be used for in microbiology?

According to the number of NGS articles reported in the Web of Science, whole genome sequencing and identification of microbial species accounted for less than 30% of all papers published using NGS (Figure 2; right panel). The other NGS studies have dealt with: (i) the discovery of new genes, small nucleotide polymorphisms (SNP), and small regulatory RNA, (ii) gene expression analysis, (iii) the examination of pooled genes from specific environments or conditions, and (iv) methods development. Hence, NGS can now be used to ask questions such as what is there, what is it doing, how does it respond to environment changes (e.g., antimicrobial treatments) and how do microbes interact with one another? Never before has a technology been used to address these questions on such a massive scale.

What will be the long-term impact of NGS on biological research?

NGS will affect biological research in three ways. First, NGS will become routine, widespread and available to small institutions and individual scientists. As a consequence, the technology will aid in the democratization of science because a scientist does not need to be associated with a genome center to conduct NGS research (9). Second, because questions/hypotheses generated by scientists depend on the availability of the technology to answer them, scientists will have to re-think old questions/hypotheses to consider them within the context of the new technology. In other words, previous questions/hypotheses based on old technology are likely to become obsolete. For example, why examine the expression of one or two genes when one can consider the expression of an entire organism? Third, in the same way that novel polymerase chain reaction (PCR) methods (e.g., quantitative PCR) arose from the invention of PCR, NGS will drive scientific innovation leading to the development of new and better ways to explore the biological world that have not yet been discovered. In other words, innovative ideas and concepts will flourish with the availability of NGS technology. Hence, it is a very exciting time to be a microbiologist!■

Current research conducted by Noble and colleagues is aimed at assessing the accuracy and precision of observed DNA target abundances in complex target mixtures using the Roche/454 pyrosequencing device at Max Planck Institute for Evolutionary Biology in Plön, Germany. Noble plans to use this technology to explore microbial communities in the human oral cavity. Dr. Noble was awarded a grant to purchase a Roche/454 GS sequencer in August, 2010 by the National Science Foundation.

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