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The Baker Lab at the OHSU School of Dentistry: leveraging bioinformatics and molecular biology to discover how the bacteria that live in our mouth impact human health and disease

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Abstract

The microorganisms living in the human oral cavity, collectively known as the oral microbiota, play a critical role in not only oral health, but systemic and overall health. The Baker Lab leverages emerging technologies in bioinformatics and molecular biology to answer fundamental questions regarding the ecology, physiology, and pathogenesis of the oral microbiota. We use a microbial 'omics approach, which has included pioneering the use of nanopore sequencing on saliva and oral bacterial RNA. The resulting work discovered novel bacterial species and biosynthetic pathways which impact the ecology of the oral microbiota and its relationship to human disease. This article will briefly define the oral microbiota. It will also summarize how bioinformatics and 'omics-based research have revolutionized oral health research. The article will then provide a broad summary of our past, present and future research and educational programs.

What is the oral microbiota/microbiome, how is it studied, and why does it matter?

The oral microbiota is the collection of microorganisms that live in the human oral cavity, and includes bacteria, viruses, archaea, and microeukaryotes such as fungi (Baker *et al.*, 2023). These microbes have a major impact on human health, with extremely prevalent and costly oral diseases such as dental caries, periodontal disease, and oral cancers having mainly microbial etiologies (Baker *et al.*, 2023). Prior to the mid-2000s, microbiological research at large, including that of the oral microbiota, was limited to the species of microorganisms that could be physically isolated, cultured (i.e., grown), and studied in a laboratory (Baker, 2023). Much of the study of microbial pathogens employed Koch's Postulates, in which causative microbial agents of disease were identified by isolation from diseased sites, grown in pure culture, and subsequently able to cause disease when introduced into healthy model organisms (Falkow, 2004). In the context of oral microbiology, these classical approaches enabled the discovery of several oral pathogens associated with oral diseases. Two prominent examples were the association of *Streptococcus mutans* with dental caries and the association of *Porphyromonas gingivalis* with periodontal disease (Edwardsson, 1968, Kagan, 1980). Organisms associated generally with good oral health, such as *Streptococcus gordonii*, were also identified and studied during this period (Nyvad & Kilian, 1990). Since one does not know *a priori* what organisms are present in an environment, or how to isolate and cultivate them, only a minority of the microorganisms residing in the oral cavity were identified and studied in-depth using traditional microbiological techniques.

The development of culture-independent analysis methods, such as high throughput sequencing and modern mass spectrometry, allowed detection and analysis of all microorganisms present in a sample, not just the ones that could be isolated and grown in a lab. This revealed that the species isolated and cultured to that point in time, using classic microbiological methods, represented a small percentage of the diversity present in most environments, including the oral microbiota (Venter *et al.*, 2004, Ley *et al.*, 2005, Gill *et al.*, 2006). In the approximately 20 years since the emergence of this technology, there has been an explosive growth of microbiome research, which has provided an ever more complete picture of which microorganisms are present or abundant in human oral microbiotas associated with good oral health, or with microbiotas associated with oral diseases such as caries, periodontal disease, and oral cancer (Baker *et al.*, 2023). In addition to its relationship with oral diseases, a growing body of evidence is also linking the oral microbiota to a myriad of systemic diseases. These include cardiovascular disease, diabetes, colorectal cancer, obesity, Rheumatoid arthritis, Alzheimer's disease and others (Hajishengallis & Chavakis, 2021, Baker *et al.*, 2023). The oral microbiota is now known to contain over 700 species, and the health-associated microbiota defends the mouth against pathogenic organisms (Chen *et al.*, 2010, He *et al.*, 2010, Baker *et al.*, 2023). As an increasing amount is known about which species are present in various environments and conditions, there is a growing need to move past this type of research and understand the mechanisms underpinning how the microbiome is affecting the health of the human host (Nascimento *et al.*, 2017, Burne, 2018).

What is 'omics research?

'Omics research is the informal term for the collective research approaches that end in the suffix, 'omics,' and each aim to characterize and quantify entire pools of molecules or interactions in a given setting. Examples that are particularly relevant to oral microbiome research are: genomics, analysis of genomes; metagenomics, simultaneous analysis of multiple genomes in a community; transcriptomics, analysis of RNA of a given species; metatranscriptomics, analysis of the RNA of a community of organisms: metabolomics, analysis of metabolites (i.e., small molecules); proteomics, analysis of proteins; lipidomics, analysis of lipids; pangenomics, analysis of gene families across multiple organisms; and phylogenomics, analysis of the evolutionary relationship between organisms. The Baker Lab has experience leveraging diverse 'omics methods across several research projects and collaborations. Examples include: (1) genomics to assemble complete genomes of novel species and strains from the oral microbiome (Baker & Edlund, 2020, Baker, 2021, Baker, 2022), (2) metagenomics to discover new species and observe disparities in the oral microbiome, and the biosynthetic pathways it encodes, associated with dental caries and periodontal disease (Aleti *et al.*, 2019, Baker & Edlund, 2021, Baker *et al.*, 2021, Baker, 2022), (3) proteomics to map the *S. mutans* acid and oxidative stress responses and discover new functions of a transcriptional regulator (Tinder *et al.*, 2022), (4) pangenomics to explore gene families across various bacterial species and strains to predict metabolic repertoires and ecological roles (Baker, 2021, Baker *et al.*, 2021, Baker, 2022), (5) phylogenomics to determine the evolutionary lineage of novel bacteria (Baker, 2021, Baker *et al.*, 2021, Baker, 2022), and (6) lipidomics to discover how bacteria modify their cell membranes in response to stress, in an effort persist and cause disease (project in progress).

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Nanopore sequencing: leveraging an emerging technology

As stated above, the development of culture-independent analysis of microbiomes was enabled by so-called next-generation sequencing (Bennett, 2004, Margulies *et al.*, 2005, Bentley *et al.*, 2008, McKernan *et al.*, 2009). Illumina was the most broadly used sequencing platform of the 2010s, and revolutionized the life sciences by reducing the cost of sequencing by orders of magnitude, providing exceptionally accurate sequencing data, and increasing throughput. However, the latest generation of emerging sequencing technologies (i.e., "third generation sequencing") is in the process of disrupting biomedical research once more (Athanasopoulou *et al.*, 2021). One technology that has gained considerable traction in the last several years is nanopore sequencing (Oxford Nanopore Technologies Inc. (ONT)) (Jain *et al.*, 2015). While Illumina sequencing-by-synthesis technology generates sequences that are generally 150 or 300 base pairs of DNA in length, ONT sequencing theoretically has no upper limit on the length of output sequence, with single reads of more than 1 million base pairs of DNA routinely reported (Jain *et al.*, 2015). Because most genomes contain repeat regions spanning much longer than 300 base pairs, the puzzle that is a given genome cannot typically be completed with Illumina short-read sequencing alone (i.e., because all these short reads match up nonspecifically to all their cognate sequences in the repeat regions of the chromosome). The long reads produced by ONT sequencing much more easily span the repeat regions of genomes enabling significantly easier assembly of complete chromosomes. However, until recently ONT technology was plagued with a considerably higher error rate than competing technologies, limiting its applicability (Amarasinghe *et al.*, 2020). This error rate decreased in recent years, with a crucial inflection point being reached in 2022, when studies illustrated that the contemporary ONT instrumentation and software could produce genome assemblies with an error rate on par with those produced by Illumina sequencing (Sereika *et al.*, 2022).

The Baker Lab was an early adapter of ONT technology, and in 2022 published the first protocols to obtain multiple complete bacterial genomes simultaneously directly from saliva using ONT sequencing (Baker, 2022). Several of the genomes completed using these methods were the first complete genome for their given species (*Candidatus* Saccharibacteria HMT-870, *Candidatus* Saccharibacteria HMT-348, *Actinomyces graevenitzii*) (Baker, 2021, Baker, 2022, Baker, 2022). Particularly intriguing were the genomes from the enigmatic Saccharibacteria family, which are essentially tiny (even by bacterial standards) parasitic bacteria that depend on larger host bacteria to survive (He *et al.*, 2015). The novel complete genomes from our study illustrated that the G6 group of Saccharibacteria likely has a different lifestyle, and possibly host and host dependencies than the more well-understood G1 group of Saccharibacteria (Baker, 2021). These differences likely extend to ecological and pathogenic roles as well, as Saccharibacteria appear to have a relationship to inflammation and periodontal disease (albeit poorly understood at this stage) (Chipashvili *et al.*, 2021). The ability to obtain complete genomes directly from complex samples, such as saliva, will revolutionize microbiology research, as it was previously only possible to obtain complete genomes of species that were isolated and cultivated in the lab in a pure culture (i.e., only incomplete, draft genomes could be obtained from metagenomes using earlier sequencing technologies) (Athanasopoulou *et al.*, 2021). Obtaining genomes that are

both complete and accurate is of importance because they then allow accurate identification and quantification of the species of interest in microbiome samples, and enable accurate prediction of the metabolic capabilities (and therefore ecological and pathogenic roles) of the species (Venter *et al.*, 2004, Naito *et al.*, 2016). This data can further guide wet-lab research and help scientists design experiments, isolate, cultivate and study species that were previously intractable (Cross *et al.*, 2019). In addition to genomics and metagenomics using ONT, The Baker Lab pioneered use of the ONT sequencing platform for RNA sequencing of oral bacteria (Baker *et al.*, 2022). RNA sequencing via ONT has several advantages as well. Because ONT can sequence native DNA and RNA molecules (unlike most sequencing methods, which must first reverse transcribe the RNA to cDNA, and/or amplify the DNA or RNA with PCR), ONT sequencing can detect base modifications, such as methylation, as well as noncanonical bases such as inosine (Garalde *et al.*, 2018, Tourancheau *et al.*, 2021, Begik *et al.*, 2022, Nguyen *et al.*, 2022). The ability to detect DNA and RNA modifications on a genome wide or transcriptome wide scale is a major advance and is likely to produce entirely new fields of microbiology research. Furthermore, the long RNA reads enable the detection of co-transcribed genes and novel RNA isoforms on a transcriptome wide scale (Garalde *et al.*, 2018, Grunberger *et al.*, 2022).

Lipidomics of bacteria, and unsaturated fatty acid production in Streptcococci

A special current emphasis of research in The Baker Lab is using lipidomics to better understand the ways in which bacteria modify their cell membranes to adapt to their environment, and in some cases, cause disease. All cells, and many viruses, have membranes, which are composed of lipid bilayers. The chemical properties of most membrane lipids render them notoriously difficult to study. As a result, lipidomics is perhaps the least utilized major 'omics discipline, and a relative deficiency exists in understanding the consequences of the lipidome in various contexts, despite certainty in its biological importance. Bacterial cells all have at least one membrane, while Gram negative organisms have two. Bacteria produce a diversity of lipids to the extent that many bacterial species can, in fact, be identified be their lipid profile alone (Abel *et al.*, 1963).

The Lactobacillales order of bacteria contains some of the most important pathogens and commensal organisms of the human microbiota. This includes the genera *Streptococcus*, *Enterococcus*, and *Lactobacillus*. Previous research has shown that a diversity of Lactobacillales increase the proportion of unsaturated fatty acids in their cell membranes in response to various environmental stresses including acid stress and oxidative stress (Fozo *et al.*, 2004). In the case of the caries pathogen, *S. mutans*, this shift to a membrane containing a greater percentage of unsaturated fatty acids was required to withstand further acid or oxidative stress, and crucially, cause disease in a rat model of dental caries (Fozo & Quivey, 2004, Fozo & Quivey, 2004, Fozo *et al.*, 2007). Our current research project on this topic seeks to address several questions and knowledge gaps raised by these observations: (1) although it is known how *S. mutans* and other Lactobacillales produce unsaturated fatty acids, it is not known how this system is regulated and controlled (i.e., how it is turned on when needed), (2) it is not known how the unsaturated fatty acids are protective, and (3) although a similar response appears to occur in all tested Lactobacillales, it is not known if it is protective and/or required for virulence in organisms other than *S. mutans* or other disease contexts. This is

a particularly important question, as Lactobacillales contains other devasting pathogens such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium*, all responsible for significant human morbidity and mortality. The bacterial lipid biosynthesis pathway is quite different at the molecular level than its eukaryotic counterpart, therefore it presents attractive targets for the development of novel antibiotics (Radka & Rock, 2022). Indeed, one of the few novel classes of antibiotics discovered and utilized in the past 30 years, triclosan, targeted the reductase step in bacterial fatty acid biosynthesis (distinct from the steps involved in unsaturated fatty acid biosynthesis) (Radka & Rock, 2022). Beyond this specific study and application, the impact of the bacterial lipidome on bacterial physiology and pathogenesis more broadly is an understudied field, with advancements in mass spectrometry technologies opening the door to lipidomics studies with a level of resolution not possible previously.

RESISFORCE: partnering Norway, Brazil, India, Canada and the U.S. to further excellence in education, research and innovation in the study of biofilms and antibiotic research.

Resistance of pathogenic microbes to antibiotics is a growing worldwide concern, with global deaths due to antibiotic resistance predicted to overtake global deaths due to cancer and become the number one cause of death worldwide by 2050 (Brown *et al.*, 2017). Our RESISFORCE project, funded by the Research Council of Norway, partners research labs from the Norwegian Institute of Public Health, University of Oslo, TATA Consultancy Services (based in Delhi, India), University of Campinas (Piracicaba, Brazil), McGill University (Montreal, Canada), University of Illinois at Chicago, Forsyth Institute and Oregon Health & Science University to engage diverse trainees, clinicians and scientists in educational outreach regarding the accelerating antibiotic resistance crisis and antibiotic stewardship. The Baker Lab has been an active partner in this project since 2019, and has co-facilitated RESISFORCE outreach symposia at dental conferences in Brussels, Belgium (CED-IADR 2021) and Marseille, France (PER-IADR 2022), as well as intensive symposia and hands-on workshops for trainees at the University of Oslo (2023) and University of Campinas (2019, 2022). Our team has also produced a massive online open course (MOOC), titled “Exploring the Landscape of Antibiotic Resistance in Microbiomes,” available on FutureLearn. This free online course enables interested clinicians, researchers, students and members of the public, to discover how antibiotic resistance has emerged as one of the most urgent public health threats, explore how the study of antibiotic resistance genes helps us understand antibiotic resistance and get hands-on experience examining data using the ResistoXplorer online tool (**www.resistoxplorer.no**—itself produced as a collaboration initiated through the RESISFORCE project (Dhariwal *et al.*, 2021)). RESISFORCE has also sponsored several international researcher exchanges between labs participating on the project, provided networking opportunities, and fostered fruitful research collaborations between the participating labs (Junges *et al.*, 2018, Junges *et al.*, 2019, Junges *et al.*, 2019, Ricomini Filho *et al.*, 2019, Salvadori *et al.*, 2019, Dhariwal *et al.*, 2021, Bajalan *et al.*, 2022, Dornelas-Figueira *et al.*, 2023, Junges *et al.*, 2023). Since dentists account for approximately 10% of all antibiotic prescriptions, and antibiotic resistance and stewardship are frequently neglected topics in dental school curriculum, the unique dentistry-focused international outreach of this program is expected to be particularly impactful (Ramanathan *et al.*, 2023).

The Baker Lab will also be hosting our four-day annual RESISFORCE symposium at OHSU on Sept. 9 - 15, 2024. This symposium will include a day of formal presentations by faculty from the RESISFORCE project, OHSU faculty in related fields, and regional leaders in microbiology and antibiotic resistance research (Sept. 13, 2024). The symposium will also feature three days of interactive workshops for dental/graduate student and postdoctoral-level trainees (Sept. 10 - 12, 2024). The workshops will include group problem-based learning sessions and presentations, working with other trainees from Brazil, Norway, U.S. and Canada, as well as a hands-on bioinformatics workshop. The symposium will be a tremendous opportunity to learn about current research in antibiotic resistance and oral health, and to network and interact on an international level with oral health researchers.

Perspective

Personalized medicine, enabling significant improvements to oral health and overall health, is on the horizon. However, an incomplete understanding of the complex oral microbiota, and its impact, continues to obstruct progress toward actionable diagnostic metrics, as well as novel therapeutic and preventative strategies. Fortunately, emerging technologies are enabling discovery in these fields at an unprecedented pace, scale and level of resolution. It is an exciting time to be at the intersection of oral health research and microbiome research.

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