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Metagenome – Inferred bacterial replication rates in cystic fibrosis airways

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ABSTRACT

Bacterial replication rates were determined from metagenome sequencing of nasal lavage, throat swabs and induced sputa collected from healthy subjects and individuals with COPD or cystic fibrosis. More than 90% of peak-to-trough coverage ratios of major clones were above 1.4 indicating that the most abundant bacterial species in the microbial communities were replicating in the airways including common inhabitants such as *Prevotella* and *Streptococcus* species as well as the cystic fibrosis pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The populations of *P. aeruginosa* and *S. aureus* were replicating their pool of chromosomes more slowly than the populations of the common inhabitants of a healthy airway microbial flora. The assessment of growth dynamics in microbial metagenomes could become a decision-making tool for the diagnosis and management of bacterial infections in cystic fibrosis.

1. Introduction

Cystic fibrosis (CF) airway microbiology has traditionally been investigated by culture-dependent procedures [1] or more lately by 16S rRNA based community profiling [2]. With next-generation sequencing technologies becoming more and more affordable, whole metagenome shotgun sequencing is getting recognized as an alternative to rDNA profiling [3]. Due to methodological limitations most microbial metagenome studies investigated CF adults with advanced lung disease who naturally expectorate comparably large volumes of respiratory secretions [4–11]. These studies resolved the complexity and/or metabolic potential of the microbial communities, but they did not address the growth dynamics of the airway microbiota whether the detected microbes are dormant or actively replicating.

Knowledge about microbial growth would be welcome for the diagnosis and management of bacterial infections. Two groups have recently developed algorithms to calculate bacterial replication rates in metagenome datasets [12,13]. Bacterial genome replication proceeds bi-directionally from the origin of replication *ori* to the terminus of replication *ter* [14]. The gradient of sequencing coverage from *ori* to *ter* can thus be exploited to determine the proportion of the bacterial population which is replicating. Here we report on our own metagenome approach to determine the replication rates of the major bacterial inhabitants in CF airways.

2. Methods

Bacterial replication rates were calculated from curated 75-bp datasets deposited in our local database [7] that compiles microbial metagenomes of nasal lavage [15], throat swabs and induced sputa [16] collected from clinically stable individuals with CF, COPD disease controls and healthy subjects. After collection, samples were immediately stored at −80 °C until DNA extraction by biochemical lysis followed by ultrasound sonication, library preparation and sequencing [7]. Bacterial clones were selected from the individual datasets that were represented by more than 100,000 curated reads in the metagenome. These datasets were re-mapped on a complete reference genome whose *ori* and *ter* loci had been identified on the chromosomal map as the turning points of the GC-skew [17]. Genome coverage was determined by read counts in 10 kb sliding windows with 5 kb overlap using the Sambamba program (https://github.com/biod/sambamba). The peak-to-trough ratio, PTR, as an estimate of bacterial growth was then determined from the ratio of sequencing coverage at the *ori* compared to the *ter* [12]. The gradient of read counts along the left and right replichore was evaluated by linear regression. To account for inflating read numbers caused by misalignments of paralogous sequences from other species or caused by strain-specific multi-copy DNA

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Table 1
Growth dynamics of prominent bacterial populations in the healthy, CF and COPD airway microbiome assessed by the ratio of sequencing coverage between the peak and trough.

Species	Condition	Source[a]	Peak-to-trough ratio PTR
P. melaninogenica	Healthy	SPU	1.47; 1.62; 1.77; 1.84; 2.01
	COPD	SPU	0.88; 1.18; 1.54; 1.81; 1.93
	CF	NAS	1.80; 1.93
	CF	THR	1.45; 1.52; 1.54; 2.01; 2.10
	CF	SPU	1.43; 1.92; 2.10
S. mitis	CF	NAS	0.98
	CF	SPU	0.98; 0.98; 1.15; 1.17; 1.23; 1.39
S. oralis	CF	SPU	1.57; 1.75; 1.98; 2.34
S. parasanguinis	CF	NAS	2.13
	CF	THR	0.94; 1.68; 1.82; 1.92; 2.19; 2.40; 2.49
	CF	SPU	1.59
S. salivarius	Healthy	SPU	1.43; 1.81; 1.93; 1.99; 2.07; 2.14; 2.09; 2.39
	COPD	SPU	1.20; 2.15; 2.17
	CF	NAS	1.55; 1.67
	CF	THR	1.41; 1.56; 1.80; 1.82; 1.86; 1.87; 1.95; 2.34
	CF	SPU	1.63; 2.11
S. aureus	CF	SPU	1.04; 1.51; 1.57; 1.61; 1.78; 1.86; 1.88
P. aeruginosa	CF	SPU	1.50; 1.77; 1.80; 1.84; 1.85; 1.94; 2.03; 2.93

[a] NAS, nasal lavage; THR, throat swab; SPU, induced sputum.

sequences, the dataset of each analyzed species in a sample was curated from outliers by Dixon's Q test. Bacteria differ in genome size and the yield of species-specific reads in a metagenome depends on the relative abundance of the species and the depth of sequencing. To allow a within-sample species-to-species comparison and/or a between-sample comparison of clones and species, the number of reads of each investigated species in a sample had to be normalized. Hence slopes derived from linear regression were normalized to an arbitrary number of 10^7 reads per 1 Mbp. This standardized parameter psr (population speed of replication) allows an unbiased comparison of the relative speed of replication in populations of bacteria which carry single *ori* and *ter* loci in their circular chromosome.

3. Results

Bacterial replication rates were determined for taxa that are most prevalent in the airways of healthy persons and patients with CF. Healthy airways are predominantly colonized with facultative and obligatory anaerobes, mainly species belonging to the genera *Prevotella*, *Rothia*, *Streptococcus* and *Veillonella* [18]. Microbial communities in CF airways are made up of variable proportions of this healthy flora and of disease-typical pathogens, most frequently *Haemophilus influenzae*, *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* [1,2,7].

We searched in our curated airway metagenome datasets for major clones of the common inhabitants of CF airways. To get robust data, a clone had to be represented by at least 100,000 curated 75-bp reads, i.e. about a 5-fold coverage of a 2-million bp genome. Table 1 lists the peak-to-trough ratios, PTR, of 78 bacterial clones retrieved from throat, upper or lower airways. A PTR value of 1.5, for example, would indicate that roughly 50% of the bacterial population is replicating 1 copy of its chromosome or – more precisely – that the number of bi-directionally moving replication forks matches the number of chromosomes in the populations. More than 90% of PTR values were above 1.4 indicating that the vast majority of the most prevalent bacterial species is replicating in healthy and CF airways. The exception was *H. influenzae* with PTR values around 1 in all examined CF samples. *H. influenzae* apparently did not grow even though it was colonizing CF patients' airways in large numbers.

Whereas PTR reflects the proportion of replicating chromosomes in a bacterial community, the newly defined parameter psr is a relative measure of the speed of replication of the bacterial population. Please note that psr like PTR is a time-independent parameter and is a relative measure of the replication rate of the bulk population of cells of a bacterial species, but psr does not provide any information about the speed of the replication machinery at the molecular level. Read counts decreased linearly from *ori* to *ter* indicating that replication is typically not synchronized in bacterial populations residing in human airways. Fig. 1 compares the raw (upper panel) and final (lower panel) psr values corrected for missing or misaligned sequences. In the CF host the populations of the CF pathogens *P. aeruginosa* and *S. aureus* were replicating their pool of chromosomes 40- to 60-fold and 3- to 9-fold more slowly than the populations of *Streptococci* and *Prevotella melaninogenica.* The common inhabitants of human airways replicated faster, but also showed a larger host-to-host variation of their population speed of replication than the two CF pathogens.

Consistent with expectation, the left and right replichores were replicated at the same average speed in *S. aureus* and *P. aeruginosa* chromosomes. In contrast, the calculated psr values of the left and right replichores were consistently different in all examined *Streptococcus* and *Prevotella* clones (Fig. 1). Provided that no interfering variable had been missed during the correction of the raw psr data, the average rate of DNA synthesis differed substantially between the two replichores. Like in *Escherichia coli*, a 'replication fork trap' should exist in *Streptococcus* and *Prevotella* species that allows the first arriving fork to enter but not to leave the terminus region [14,19]. The unequal speed of the two replichores in *Streptococci* and *Prevotella* was unexpected, but becomes comprehensible in the light of recent findings on *E. coli* replisomes [14] that bacterial polymerases exchange at replication forks [20,21] and that leading-strand and lagging-strand DNA synthesis can be carried out in a decoupled and stochastic way, in which both polymerases and helicase work independently [22].

4. Discussion

Replication rates of bacterial chromosomes have initially been deduced from cell doubling times [23] and more lately by pulse labelling [24]. Alternatively, the growth of bacterial populations can be inferred from deuterium labelling of biomarker molecules, for example, the growth rates of populations of *S. aureus, P. melaninogenica* and *Stenotrophomonas maltophilia* in CF sputum were determined from isotopic enrichment in species-specific fatty acids by mass spectrometry [25,26]. These approaches assay a single or few species of interest, whereas metagenome sequencing can in principle assess all bacteria provided that the number of reads is sufficient for analysis.

In situ bacterial growth rates in metagenomic cohorts have so far been evaluated in the gut, skin and mouth of infants [13,27], the vagina during pregnancy [28] and in the oral cavity and gut of adults [12] who participated in the Human Microbiome Project. In our metagenome study in healthy and CF airways the median PTR of the major bacterial species was determined to be 1.82 (Table 1) which is higher than that of 1.4 and 1.5 reported for infant [13] and adult [12] gut microbiomes implying that bacterial communities are growing faster in airways than in the intestine.

We chose a conservative approach to evaluate bacterial replication rates only for major clones with high genome coverage in the metagenome datasets in order to minimize false alignments, copy number variations and variable coverage levels. This procedure is robust but comes with a loss of information about the potentially different growth dynamics of minor clones. This problem should be overcome in the foreseeable future by the emerging third-generation sequencing technologies of single molecules. The generation of longer strain-specific reads will facilitate less error-prone assemblies and alignments and thus will allow the calculation of more reliable PTR and psr values. Then the assessment of growth dynamics in microbial airway metagenomes could become a routine procedure for the monitoring of microbial communities in CF airways.

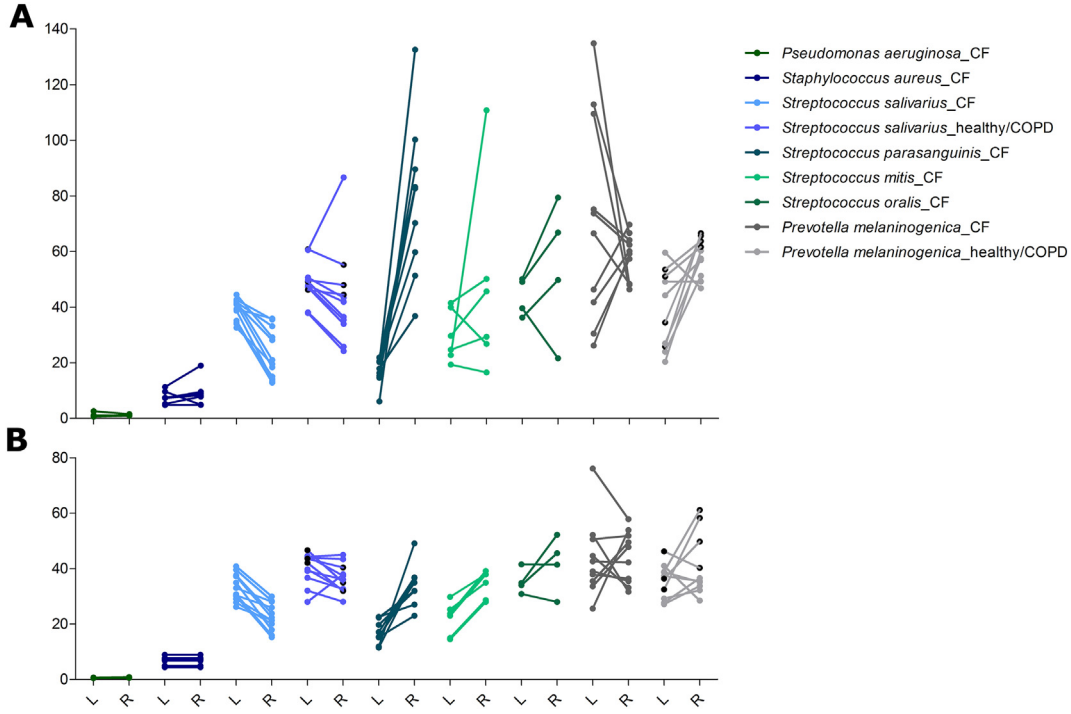

Fig. 1. Population speed of replication (psr) of dominant bacterial clones in the microbial communities of oropharynx, upper and lower airways of healthy subjects, individuals with COPD and patients with CF. Psr values were determined from linear regression of the gradient of read coverage from *ori* to *ter*. The psr values of the upper panel A were calculated from all reads mapped onto a completely sequenced reference genome of the species whereas the psr values of the lower panel B are based on the datasets that were curated from 10 kb genomic segments with very low or very high read coverage. Within the 'healthy/COPD' group the bacterial clones retrieved from patients with COPD are marked with a black dot; the other clones were retrieved from respiratory secretions of healthy probands. Please note that the population speed of replication of left (L) and right (R) replichore was different for most *Streptococci* and *Prevotella* clones.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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