Microbes rarely exist as single species planktonic forms as they have been commonly studied in the laboratory. Instead, the vast majority exists as part of complex polymicrobial biofilm communities attached to host and environmental surfaces. Mycobacterial tuberculosis (MBT) is no exception. A number of researchers have shown that in the experiment in vivo model, MBT can form biofilm-like structures in the lungs.

The aim of the study is to demonstrate the role of tuberculosas satellite microbiota as example of polymicrobial biofilm existent in a lung of TB patients. Our study of clinical MBT strains shown less 5% of them were able to produce mature biofilms (pellicle) on a liquid medium. Although we might expect that pathogenic MBT could gain obvious advantage in case of growth in necrotic foci in lungs and it should keep this ability in the first passage in vitro. It was found feature of MBT strains produced pellicle on liquid medium to grow on Levinstein—Jensen by specific R colonies. It looks as disk with a convex center, “UFO-colonies”. It was shown on in vitro model that about of 50% clinical MBT strains can coexist together with Bacillus licheniformis, also isolated from sputum of TB patient. Moreover, after pellicle formation by bacilli in the first 3 days, the growth of MBT was continued for next 30 days under the bacillary pellicle. It is very important that investigated bacilli had a high tolerance to streptomycin, ethionamide, isoniazid and ethambutol, e.i. to four of the 12 basic anti-TB drugs.

The study on 16S rRNA metagenomic and massively parallel sequencing (NGS) DNA of several tuberculomas was conducted. It was shown that quantity of MBT genomes were less 5% in all cases. The vast majority species belonged to Gram-positive Firmicutes like Staphylococcaceae and also a small amount of Gram-negative taxa was found.

We can assume that anti-tuberculosis therapy is confronted with not only MBT, but with polymicrobial biofilm communities, which formed by the etiological agents of tuberculosis and also by a large number of other satellite microorganisms in lungs. It is very important that this microbial community in TB-patient lungs of should form a cumulative resistance to anti-tuberculosis therapy during long-term treatment. We can expect that the cumulative resistance of a polymicrobial biofilm in the TB-patient lungs may be significantly differing from the resistance of detected in the clinical laboratory TB strains.

BACTERIAL WGS AND HOST GENOME-WIDE SNP ANALYSIS OF TUBERCULOSIS PATIENTS IN THAILAND

P. Palittapongarapin1, S. Mahasirimonkol2, K. Tokunaga3
1Department of Microbiology, Faculty of Science, Mahidol University and the National Science and Technology Development Agency, Bangkok, Thailand; 2Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand; 3University of Tokyo, Tokyo, Japan

Mycobacterium tuberculosis has been a human pathogen for a long time, providing ample opportunities for genomic interactions between the two organisms. Evidences of co-evolution has been reported. We have performed genomic studies in a cohort of tuberculosis patients in Chiangrai, northern Thailand. The genomes of M. tuberculosis isolated from 1170 patients during 2003–2010 were sequenced. The genomes of the same patients were also evaluated using high-density SNP arrays. The bacteria were genetically heterogeneous, with majority belonging to various sublineages of lineages 1 and 2. Refinement of classification of lineage 1 were proposed and a few novel sublineages of the others were identified especially in remote populations. The patients mostly belonged to three genetic groups, identified by principal component analysis, and three self-identified ethnicity groups. The profiles of patients infected by sublineages varied especially among sublineages of lineage 2. There were strong correlations between the bacterial genotypes and human ethnicity. GWAS identified a few genes associated with particular genotypes of the bacteria. Together with historical records, this study indicated that both the founder effects and co-evolution may explain the associations. This study provided some insights to the bacterial host interactions and useful information for the development of vaccines and other control measures for tuberculosis and is being replicated in a cohort of 600 patients in 2016–2018 with some patients studied by WGS.