Rapid detection of mycobacterial growth in in vitro cultures by VOC analysis

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Introduction

Ambient air often contains thousands of different volatile organic compounds (VOCs). These VOCs are increasingly studied as potential biomarkers for various applications, such as diagnosis of diseases or detection of bacterial growth. In a study with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) as model organism, we investigated if bacterial growth in in vitro cultures can be detected by analyzing VOCs in the headspace. Especially if it is possible already after 1 week of cultivation.

Methods

The headspace of MAP in vitro cultures (n=8) was analyzed 1,2,3,4 and 6 weeks after inoculation with a GC-differential-ion-mobility-spectrometer (SIONEX). For comparison, different culture media with no bacterial growth (n=12) were included (pure(Aa), sterile-filtered(Ba), heat-inactivated(Ca)). Headspace was collected with a disposable PTFE tube and the drawn room air was filtered with a multi-stage filter (Figure 1). The spectra were analyzed by a statistical program based on cluster analysis.

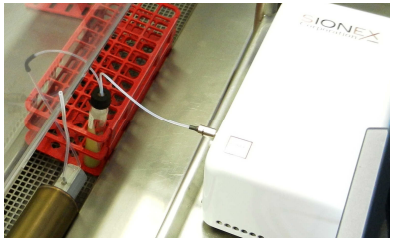

Figure 1: Sionex DMS with connected breeding ground and filter

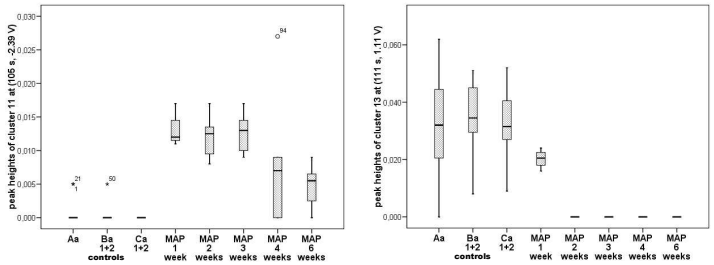

Figure 2: Boxplots of intensities of two example VOCs (no bacterial growth (Aa, Ba, Ca) versus different aged MAP cultures)

		Actual Group	Predicted Group Membership						Total
			Aa/Ba/Ca	MAP-1W	MAP-2W	MAP-3W	MAP-4W	MAP-6W	
Cross-Validated (%)		Aa/Ba/Ca	96.7	.0	.0	.0	.0	3.3	100.0
		MAP-1W	.0	100.0	.0	.0	.0	.0	100.0
		MAP-2W	.0	.0	100.0	.0	.0	.0	100.0
		MAP-3W	.0	.0	.0	100.0	.0	.0	100.0
		MAP-4W	.0	12.5	12.5	.0	75.0	.0	100.0
		MAP-6W	.0	.0	.0	.0	12.5	87.5	100.0

Table 1: Cross-validated classification of all measured probes (no bacterial growth (Aa, Ba, Ca) versus different aged MAP cultures; 96 measurements in total)

Results

Cluster analysis resulted in 160 independent clusters; each representing a specific VOC. The headspace of bacterial in vitro cultures showed highly significant differences in peak intensities ($p<0.001$, all time points) to the headspace of culture media without growth for 10 of the 160 clusters. These differences already showed up after just one week (examples seen in Figure 2). A cross-validated discriminant analysis resulted in a correct classification rate of 94.8 %. Whereas false classifications between positive and negative samples did just occur for 6 week old cultures (Table 1).

Discussion

The study showed that VOC analysis of headspaces via GC-IMS/DMS has the potential to be a very fast and reliable method to detect bacterial growth in in vitro cultures. Furthermore this method is very safe, because it is not necessary to reopen the samples. For MAP, growth could be detected just one week after inoculation, which is much faster than currently used methods which take at least 4 to 6 weeks. An identification of the relevant VOCs for detecting MAP-growth would be interesting and is still pending, but is not needed for applying this method since it will not improve the correct classification rates of new unknown samples.

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