A nutrient supplement promotes the rapid detection and sensitivity of mycobacteria in clinical samples via the differential regulation of dormancy genes and ncRNAs

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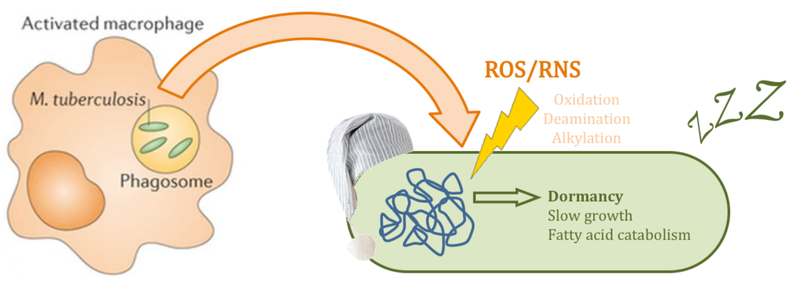
INTRODUCTION

Mycobacterium tuberculosis (*Mtb*) is the intracellular pathogen that causes tuberculosis (TB), a deadly human disease that kills millions of people every year[1]. The success of *Mtb* is mainly related to its capacity to switch from a replicative to a dormant state. This mechanism is referred to as dormancy, causing a latent infection, during which the bacteria are viable but non-replicating and therefore less susceptible to treatment. Efficient detection and cultivation of *Mtb* are crucial for rapid diagnosis and optimal treatment of TB in the era of rampant multidrug-resistant *Mtb* strains[2,3]. Mycobacterial culture still remains the "gold standard" for *Mtb* complex (MTBC) and nontuberculous mycobacteria (NTM) diagnostics and is required to perform complete drug susceptibility testing, epidemiology, and in-depth surveillance.



PROJECT OUTLINE

Clinical samples

1635 clinical samples

Decontamination
NALC

Time to detection (TTD)

+/- Supplement

MGIT with Middlebrook 7H9 broth

BACTEC MGIT 960 system

Identification of mycobacterial species
Cobas TaqMan 48 analyser, GenXpert MTB/RIF kit, 16S rRNA gene

Transcriptomic profiling

CULTURE SUPPLEMENT
1day, 7days

Total RNA isolation & High-throughput RNA-seq
mirVana miRNA isolation kit | Illumina HiSeq-2000 sequencer

Quality control: fastQC, FASTX-toolkit
Read alignment and counting: segemehl.x, HTSeq [4]

Differential expression analyses → DESeq2[5]
Presence /Absence analyses → Rockhooper, COV2HTML

mRNAs encoding ORFs
ncRNAs → Prediction of **Potential targets** → intaRNA

Selected candidates
Validation
qPCR
Nothern blot → mirVana miRNA Probe construction kit

METHODS

Clinical samples | 1635 human clinical specimens were collected from patients suspected of contracting TB or NTM infections at Oslo University Hospital, Rikshospitalet. The samples were decontaminated and cultured in Middlebrook 7H9 broth in the BACTEC MGIT 960 system according to the standard TB diagnosis protocol, but with or without the addition of a growth supplement. Time-to-detection (TTD) was recorded, and identification of the mycobacterial species were conducted using various PCR techniques.

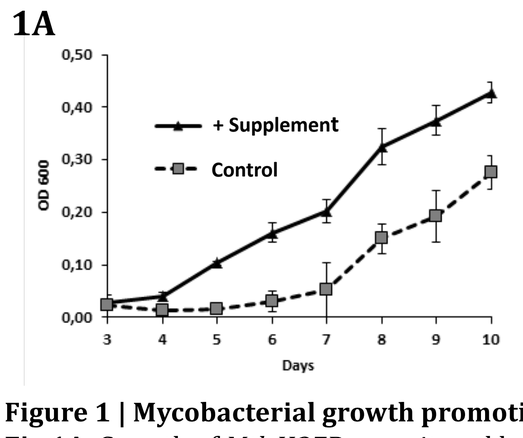
Transcriptomic profiling | *Mtb* reference strain H37Rv was cultivated with and without defined culture supplements. Total RNA was isolated and subjected to high-throughput RNA-seq. 49 non-coding RNAs (ncRNAs) were identified using Rockhopper and their potential mRNA targets were predicted using intaRNA. A subset of the ncRNAs differentially transcribed in the presence/absence of the supplement was selected for validation by northern blot.

GROWTH EFFECT

Group	Culture positive samples		Increased sensitivity	Time gain (+ Supplement)
	+	-	+	Days - average (range)
MTBC	216	167	22.70%	5 (1-42)
NTM	93	57	38.70%	3.7 (2-62)

Table 1 | Increased sensitivity after addition of nutrient supplement. Effect of the addition of nutrient supplements on the sensitivity of mycobacterial detection by culture and reduction of TTD of mycobacteria from clinical specimens.

1A



1B

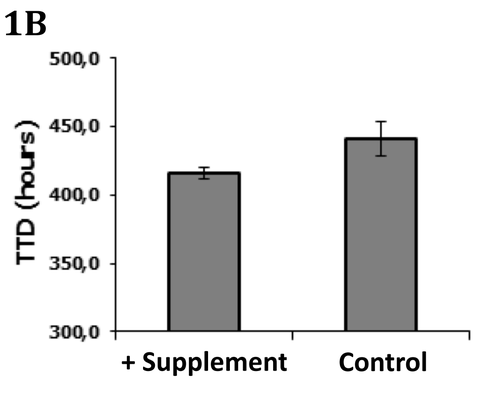


Figure 1 | Mycobacterial growth promotion.
Fig 1A. Growth of *Mtb* H37Rv monitored by OD_{600} measurement at indicated time points in the presence of the supplement (solid line) or phosphate-buffered saline (PBS) (stippled line). The experiment was conducted in triplicates, and was repeated three independent times. Error bars depict standard deviations (SD) from triplicates.
Fig 1B. Growth of *Mtb* H37Rv (≈5 cfu) in MGIT in the presence of culture supplement or PBS was monitored in BACTEC MGIT 960 system. TTD was recorded from the positive output. The error bars depict SD of TTD (hours) from quadruplicates.

AIM OF THE PROJECT

1. To increase the sensitivity and reduce the time to detection of *Mtb* and NMT from clinical specimens by optimizing liquid culture media for use in routine diagnostic procedure.
2. To study the mode of action of the nutrient supplements in promoting the growth of *Mtb* and the sensitivity of *Mtb* detection.

mRNAs

2

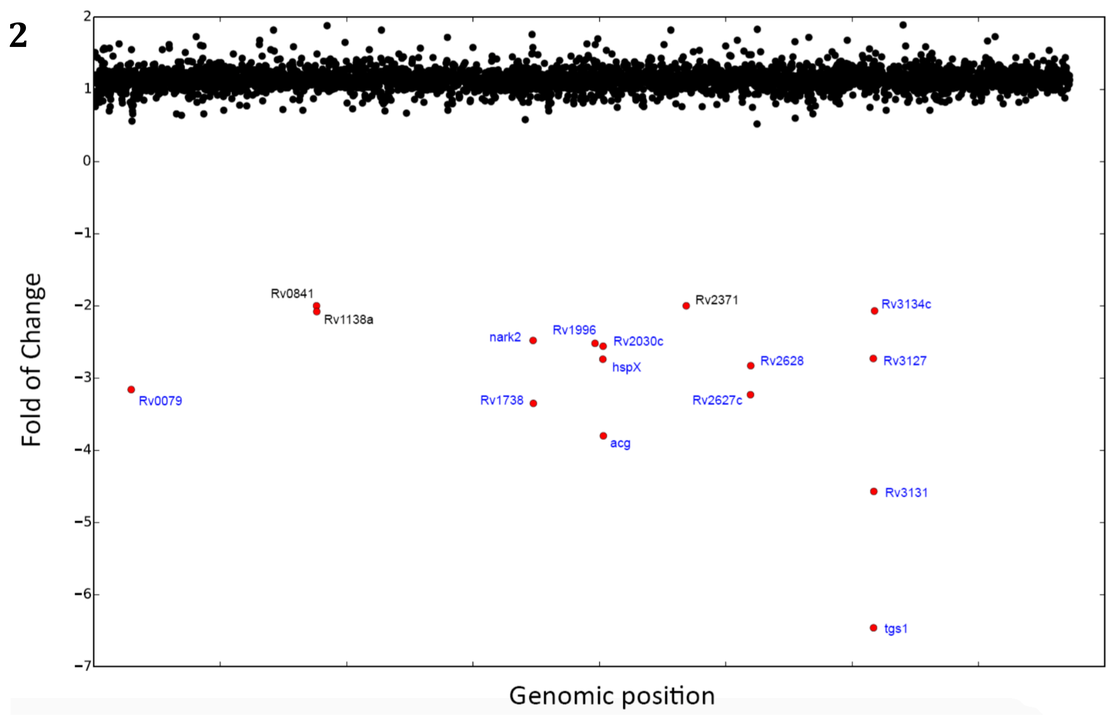
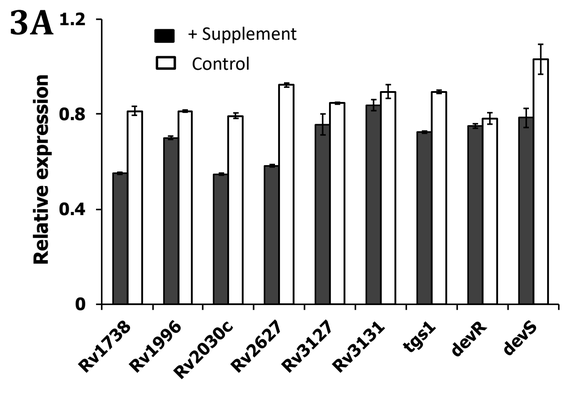


Figure 2 | Nutrient supplement down-regulates the expression of dormancy genes in *Mtb*. Genes with a fold change of ≥2 are represented by red circles. Genes indicated in blue belong to the DosR regulon.

3A



3B

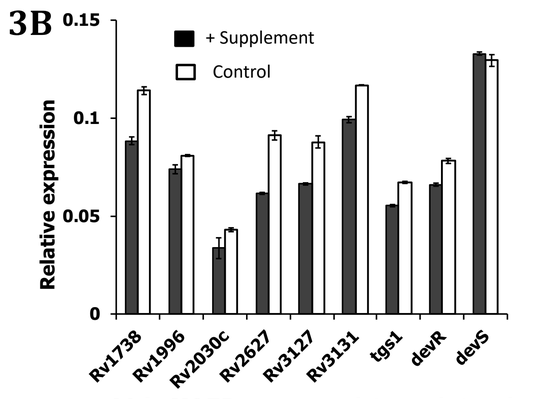


Figure 3 | Gene expression of dormancy genes. *Mtb* H37Rv was cultivated in the presence of Supplement or PBS (control) in shake flasks for 1 day (**3A**) and 7 days (**3B**). The relative standard curve method was used to calculate the mRNA levels, and the expression of the genes was normalized to the expression of *sigA*. An untreated sample was used as the calibrator. The independent cultures were analysed three times in duplicate and the error bars indicate the mean ± SD from three repeats.

sRNAs

4

5A

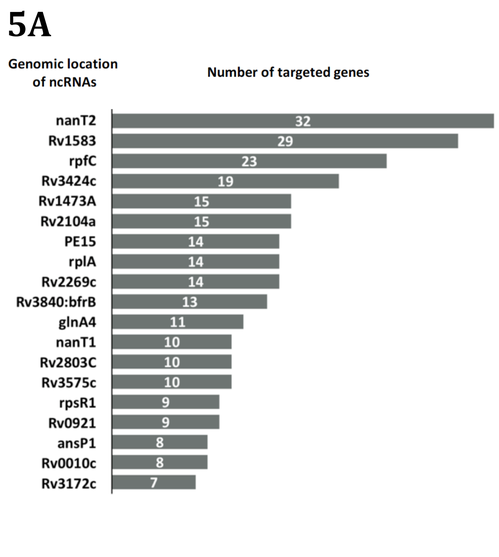
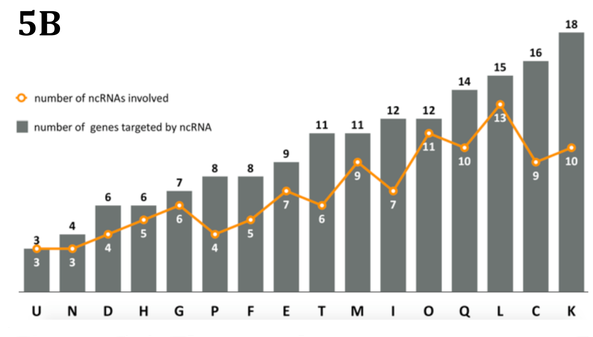


Figure 4 | Genomic distribution of identified sRNAs and their predicted targets. The circle represents the *Mtb* genome. Lines connect sRNAs with their predicted targets.

5B



[U] Intracellular trafficking, secretion, and vesicular transport
[N] Cell motility
[D] Cell cycle control, cell division, chromosome partitioning
[H] Coenzyme transport and metabolism
[G] Carbohydrate transport and metabolism
[P] Inorganic ion transport and metabolism
[F] Nucleotide transport and metabolism
[E] Amino acid transport and metabolism
[T] Signal transduction mechanisms
[M] Cell wall/membrane/envelope biogenesis
[I] Lipid transport and metabolism
[O] Post-translational modification, protein turnover, and chaperones
[Q] Secondary metabolites biosynthesis, transport, and catabolism
[L] Replication, recombination and repair
[C] Energy production and conversion
[K] Transcription

Figure 5 | The supplement represses ncRNAs involved in transcription, energy metabolism, and DNA metabolism. The putative targeted regions for each ncRNA identified were predicted using intaRNA (**5A**). Subsequently, the genes targeted by these ncRNAs were classified according to the COG classification system (**5B**).

CONCLUSIONS

- The growth supplement shortened the TTD of *Mtb* and NTM species by an average of 5 days and 3.7 days, respectively. It also increased the sensitivity by 22.7% for *Mtb* specimens and 38.7% for NTM specimens.
- NGS of RNA showed the differential expression of 16 coding transcripts. Of these, 13 belonged to the *Mtb* dormancy regulon.
- 49 sRNAs (37 novel) were differentially regulated and were predicted to target genes playing a role in energy production and carbohydrate transport and metabolism.

Adding the culture supplement thereby provided sensitive and rapid TB diagnostics, relevant for any clinical mycobacteriology laboratory.

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