Published in final edited form as:
Methods Mol Biol. 2018 January 01; 1736: 117–128. doi:10.1007/978-1-4939-7638-6_11.

Transcriptional profiling *Mycobacterium tuberculosis* from patient sputa

Leticia Muraro Wildner[1,2], **Katherine A Gould**[3], **Simon J Waddell**[2,*]

Leticia Muraro Wildner: leticiamw@gmail.com; Katherine A Gould: kgould@sgul.ac.uk

[1]Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

[2]Department of Global Health and Infection, Brighton and Sussex Medical School, University of Sussex, Brighton, BN1 9PX, UK

[3]Institute for Infection and Immunity, St George's University of London, London, SW17 0RE, UK

Summary

The emergence of drug resistance threatens to destroy tuberculosis control programmes worldwide, with resistance to all first-line drugs and most second-line drugs detected. Drug tolerance (or phenotypic drug resistance) is also likely to be clinically relevant over the 6-month long standard treatment for drug-sensitive tuberculosis. Transcriptional profiling the response of *Mycobacterium tuberculosis* to antimicrobial drugs offers a novel interpretation of drug efficacy and mycobacterial drug-susceptibility that likely varies in dynamic microenvironments, such as the lung. This chapter describes the non-invasive sampling of tuberculous sputa and techniques for mRNA profiling *M.tb* bacilli during patient therapy to characterise real-world drug actions.

Keywords

Mycobacterium tuberculosis; Mycobacteria; Transcriptional profiling; Transcriptome; Sputum; RNA extraction; RNA Amplification; Microarray analysis

1 Introduction

Transcriptional profiling is an approach that can assist in understanding how cells respond to their changing environment. Gene expression profiling has been applied to *Mycobacterium tuberculosis* to define adaptations to: antimicrobial drug exposure *in vitro* [1,2]; the changing macrophage intracellular environment [3–5]; and animal models of disease [6]. Genome-wide mRNA patterns have also captured snapshots of human host-pathogen interplay from expectorated sputa [7] or lung resection tissue [8]. More recently, transcriptional profiling bacilli from sputa has allowed *M.tb* responses to standard regimen drug therapy to be mapped in a clinical setting, revealing insights into the physiological state of *M.tb* expectorated from the lungs and understanding drug efficacy in patients [9,10]. Multiple techniques exist for mapping mRNA on a genome-wide or near genome-wide scale from quantitative RT-PCR panels and multiplex detection methodologies, to microarrays, to

*Corresponding author: s.waddell@bsms.ac.uk.

Europe PMC Funders Author Manuscripts

RNAseq using the range of next-generation sequencing platforms. Microarray hybridisation as an established technology continues to be useful in some settings. For example, the sequence specificity of target-probe hybridisation allows a genome-wide profile to be generated from samples against a background of other RNAs, such as *M.tb* bacilli recovered from human sputum.

This chapter describes the isolation and purification of mycobacterial RNA from human expectorated sputa using a differential lysis technique, followed by RNA amplification using a modified Eberwine *in vitro* transcription method. The amplified RNA is then chemically labelled with fluorophore using non-enzymatic technology and hybridised to microarrays, designed by the Bacterial Microarray Group at St George's and manufactured by Agilent Technologies.

2 Materials

2.1 Mycobacterial RNA Extraction and Amplification

1. 5M GTC solution: 5 M guanidine thiocyanate, 0.5 % w/v sodium N-lauryl sarcosine, 25 mM sodium citrate pH7, 1 % v/v Tween-80, 0.1 M β-mercaptoethanol (*see* Note 1).
2. 30 mL V-bottom universal tubes (*see* Note 2).
3. Benchtop centrifuge.
4. TRIzol® reagent (Thermo Fisher Scientific) (*see* Note 3).
5. 2 mL screw-capped tubes with O-rings containing 0.1 mm silica beads (Lysing matrix B, MP Biomedicals).
6. Reciprocal shaker (FastPrep-24, MP Biomedicals) or equivalent.
7. 1.5mL nuclease-free tubes.
8. Microcentrifuge.
9. Chloroform (molecular grade).
10. Isopropanol (molecular grade).
11. Ethanol (molecular grade) 100% and 70%.
12. RNase-free water.
13. RNeasy® mini columns (Qiagen).
14. RNase-free DNase kit (Qiagen).
15. Nanodrop Spectrophotometer (Thermo Fisher Scientific).
16. Agilent 2100 Bioanalyser system (Agilent Technologies).
17. MessageAmp™ II-Bacteria RNA amplification kit (Thermo Fisher Scientific)
18. PCR thermal cycler, heat block or incubator.
19. Vortex mixer.

2.2 Sample Labelling and Microarray Hybridisation

1. Amber-coloured tubes, 0.5 mL and 1.5 mL (Alpha Laboratories).
2. Nuclease-free water.
3. Kreatech Universal Linkage System (ULS™) Fluorescent Labelling Kit for Agilent microarrays with Cy3 and KREApure columns (Leica Biosystems).
4. PCR thermal cycler.
5. Heat block.
6. Oven set at 37°C.
7. Microcentrifuge.
8. Vortex mixer.
9. RNA Fragmentation Reagents (Thermo Fisher Scientific).
10. Hi-RPM Hybridisation Buffer (2x) (Agilent Technologies).
11. Gene Expression Wash Kit (Gene Expression Wash Buffer 1, Gene Expression Wash Buffer 2, 10% Triton X-102) (Agilent Technologies).
12. Hybridisation gasket slide kit (8 microarrays per slide format) (Agilent Technologies).
13. Microarray slides (Agilent eArray 60-mer SurePrint HD format, Agilent Technologies) in this case a 8x15k *M.tb* complex pan-genome microarray generated by the Bacterial Microarray Group at St. George's (ArrayExpress accession number ABUGS-41) [11,10].
14. Microarray hybridisation chamber (Agilent Technologies).
15. Microarray hybridisation oven with rotator rack (Agilent Technologies).
16. Glass slide-staining trough (x3) with swing handle slide rack (x1).
17. Magnetic stir plate and magnetic stir bar (x2).
18. Ozone-barrier slide cover kit (Agilent Technologies).
19. Agilent Microarray Scanner; G4900DA, G2565CA or G2565BA (Agilent Technologies) with Agilent Feature Extraction Software.

3 Methods

3.1 Mycobacterial RNA Extraction and Amplification

1. Immediately after expectoration, add the patient sputa to 4 volumes of 5M GTC solution and mix. Aliquot the sputa/GTC mixture into 30 mL universal tubes and spin at 1,800 *g* in a benchtop centrifuge for 30 minutes (*see* Notes 4 and 5).
2. Remove the supernatant. Combine sample pellets (if using multiple tubes per sample) in approximately 15 mL GTC solution, washing the universals with GTC solution to ensure all bacilli are recovered. Spin in a single universal tube

per sample at 1,800 *g* in a benchtop centrifuge for 20 minutes and remove the supernatant.

3. Re-suspend the pellet in 1 mL TRIzol and transfer the suspension to a 2 mL screw-capped tube containing 0.5 mL of 0.1 mm silica beads. Wash the universal tube with an additional 200 μL TRIzol to recover all bacilli in 1.2 mL final volume (*see* Notes 6 and 7).

4. Lyse the bacteria using a reciprocal shaker at speed 6.5 for 45 seconds, then incubate at room temperature for 10 minutes.

5. Add 200 μL chloroform to each sample, vortex for 30 seconds, then incubate at room temperature for 10 minutes to partition the aqueous and phenolic phases. Centrifuge at 15,000 *g* in a microcentrifuge for 15 minutes at 4°C.

6. Transfer the aqueous phase to a new 1.5 mL tube, add an equal volume of chloroform and centrifuge at 15,000 *g* in a microcentrifuge for 15 minutes at 4°C.

7. Transfer the aqueous phase to a fresh nuclease-free 1.5 mL tube, add 0.8 volume of isopropanol and mix by inverting. Incubate overnight at -20°C to precipitate the nucleic acids (*see* Note 8).

8. Centrifuge the samples in a microcentrifuge at 15,000 *g* for 20 minutes at 4°C to pellet the nucleic acid (*see* Note 9).

9. Remove the supernatant carefully by pipetting and wash the pellet with 500 μL cold 70% ethanol. Centrifuge in a microcentrifuge at 15,000 *g* for 15 minutes at 4°C.

10. Remove the ethanol by pipetting, re-spin the tubes briefly and remove any additional liquid.

11. Air-dry the pellet for 5-10 minutes at room temperature and re-suspend in 100 μL RNase-free water. Store briefly on ice and proceed to RNA clean-up using RNeasy Mini Columns, with buffers prepared and stored according to manufacturer's instructions.

12. Add 350 μL RNeasy RLT buffer to each 100 μL sample and mix thoroughly by pipetting.

13. Add 250 μL 100% ethanol, mix thoroughly by pipetting and apply immediately to an RNeasy Mini Column placed in a 2 mL collection tube. Centrifuge in a microcentrifuge at 9,000 *g* for 15 seconds, discard the flow-through.

14. Apply 350 μL RNeasy RW1 buffer to the column, centrifuge in a microcentrifuge at 9,000 *g* for 15 seconds and discard the flow-through.

15. DNase I treat the samples to remove contaminating DNA, using the Qiagen RNase-free DNase kit. Apply 80 μL of DNase I: buffer RDD mix (10 μL DNase I, 70 μL RDD buffer, prepared immediately before use) directly onto the column matrix. Incubate at room temperature for 15 minutes.

16. Wash the column with 350 µL RW1 buffer and centrifuge in a microcentrifuge at 9,000 *g* for 15 seconds. Discard the flow-through.

17. Wash the column with 500 µL RPE buffer, centrifuge in a microcentrifuge at 9,000 *g* for 15 seconds. Discard the flow-through.

18. Pipette 500 µL RPE buffer onto the column matrix and centrifuge at 9,000 *g* in a microcentrifuge for 2 minutes. Place the column into a fresh 2 mL collection tube and centrifuge in a microcentrifuge for an additional 1 minute at 15,000 *g* to prevent carry-over of wash buffer.

19. Transfer the column to a fresh nuclease-free 1.5 mL tube and add 30 µL RNase-free water directly onto the column matrix. Incubate at room temperature for 2 minutes and centrifuge at 9,000 *g* in a microcentrifuge for 1 minute to elute the RNA. Re-apply the eluate to the column, incubate for further 2 minutes and centrifuge at 9,000 *g* for 1 minute (*see* Note 10).

20. Quantify the RNA samples and assess the integrity of the RNA using the Nanodrop Spectrophotometer and Agilent 2100 Bioanalyser (or similar) following manufacturers' instructions.

21. Store the samples at -70°C (*see* Note 11) or continue with amplification of the RNA using the Bacteria MessageAmp II system.

22. Adjust RNA sample volume to 5 uL with nuclease-free water (*see* Note 12). Incubate for 10 minutes at 70°C, before placing on ice for 3 minutes. Briefly centrifuge, then add 5 uL polyadenylation master mix (1 uL 10x buffer, 1 uL RNase inhibitor, 0.5 uL ATP, 1 uL PAP, 1.5 uL nuclease-free water) and incubate at 37°C for 15 minutes. Place on ice before proceeding immediately to the next step.

23. Add 10 uL reverse transcription master mix (1 uL 10x first strand buffer, 1 uL T7 oligo-dT, 4 uL dNTP mix, 1 uL ArrayScript reverse transcriptase, 3 uL nuclease-free water), mix gently by pipetting and incubate at 42°C for 2 h. Place the reactions on ice, then centrifuge briefly.

24. Add 80 uL second strand master mix (10 uL 10x second strand buffer, 4 uL dNTP mix, 1 uL RNase H, 2 uL DNA polymerase, 63 uL nuclease-free water), mix by pipetting then incubate at 16°C for 2 h. Return to ice, centrifuge briefly.

25. To purify the cDNA, add 250 uL cDNA binding buffer to each sample, and mix by pipetting. Transfer the samples onto the cDNA filter cartridge matrix and centrifuge at 9,000 *g* for 1 minute in a microcentrifuge. Discard the flow-through. Wash with 500 uL wash buffer and centrifuge at 9,000 *g* for 1 minute. Discard the flow-through and then centrifuge the columns for an additional minute to remove excess wash buffer. Transfer the filter cartridges into clean cDNA elution tubes, and elute by adding 18 uL preheated-55°C nuclease-free water to the column matrix. Incubate at room temperature for 2 minutes then centrifuge at 9,000 *g* for 1.5 minutes.

26. Add 24 uL unmodified *In Vitro* Transcription (IVT) master mix (4 uL 10x reaction buffer, 4 uL T7 ATP, 4 uL T7 CTP, 4 uL T7 GTP, 4 uL T7 UTP, 4 uL T7 enzyme) to each sample (total reaction volume 40 uL), mix gently, and incubate at 37°C for 16 h (*see* Note 13). After incubation, make up the sample volume to 100 uL by adding 60 uL nuclease-free water. Place on ice.

27. To purify the amplified RNA (aRNA), add 350 uL aRNA binding buffer to each sample and mix by pipetting. Add 250 uL 100% ethanol and mix by gently pipetting. Transfer onto the aRNA filter cartridge matrix and centrifuge at 9,000 *g* in a microcentrifuge for 1 minute. Discard the flow-through.

28. Wash the columns with 650 uL Wash buffer, before centrifuging at 9,000 *g* for 1 minute. Discard the flow-through and re-centrifuge the columns at 9,000 *g* for an additional minute to remove excess buffer.

29. Transfer the filter cartridges into fresh aRNA elution tubes. Elute the aRNA by adding 50 uL preheated-55°C nuclease-free water, incubate at room temperature for 2 minutes and centrifuge for 1.5 minutes at 9,000 *g*. Repeat elution a second time with a further 50 uL nuclease-free water. Estimate aRNA yield using the Nanodrop spectrophotometer, and store aRNA at -70°C.

3.2 Sample Labelling and Microarray Hybridisation

1. Sample labelling using the non-enzymatic Kreatech Universal Linkage System (ULS). For each sample, add 1 μg aRNA, 1 μL ULS-Cy3, 1.5 μL 10x Labelling solution and adjust volume to 15 μL. Mix by pipetting and incubate at 85°C for 15 minutes (*see* Notes 14 and 15).

2. Transfer the samples to ice and incubate for 3 minutes. Centrifuge briefly.

3. Remove non-reacted ULS-Cy3 using KREApure columns. Resuspend the KREApure column material by briefly mixing using a vortex mixer.

4. Loosen cap ¼ turn, snap off the bottom closure and place the column into a 2 mL collection tube.

5. Centrifuge the column at 15,000 *g* in a microcentrifuge for 2 minutes, and discard the flow-through and cap. Place the column back into the same collection tube.

6. Add 300 μL nuclease-free water to the column and spin for 2 minutes at 15,000 *g*. Discard the flow-through and collection tube, and transfer the column to a new 1.5 mL tube.

7. Add labelled aRNA to the column matrix and centrifuge for 2 minutes at 15,000 *g* in a microcentrifuge. Optional, use 1.5 μL of each sample eluate to measure Cy3-incorporation using the Nanodrop Spectrophotometer.

8. Transfer the aRNA to a 0.5 mL nuclease-free tube and fragment by adding 1.5 μL 10x fragmentation buffer. Incubate at 70°C for 15 minutes.

9. Centrifuge briefly and add 1.5 μL stop solution, mix by pipetting and place on ice.

10. Briefly centrifuge. Prepare the hybridisation solution adding 11.3 μL labelled aRNA, 11.2 μL KREAblock blocking agent and 22.5 μL Agilent 2x Hybridisation buffer to a fresh 0.5 mL tube. Mix thoroughly (by vortexing) being careful not to introduce bubbles (*see* Note 16).

11. Place a clean gasket slide (to match microarray layout, in this instance 8x 15k) into the hybridisation chamber base (*see* Note 17).

12. Slowly dispense 40 μL hybridisation solution onto the gasket well in a “drag and dispense” manner. Do not allow the liquid to touch the edges of the gasket well and try not to introduce bubbles while pipetting. Load the rest of the samples into the remaining gasket wells (*see* Notes 18 and 19).

13. Place the active side of the microarray slide face down onto the gasket slide (numeric barcode facing up, Agilent-labelled barcode facing down) (*see* Note 20).

14. Add the hybridisation chamber cover, slide the clamp into place and hand tighten.

15. Rotate the assembled hybridisation chamber to check that the air bubble in each well of the gasket moves the sample across the microarray surface. Tap to move stationary air bubbles if necessary (*see* Note 21).

16. Place the hybridisation chamber into the rotator rack of the hybridisation oven set to 65°C. Rotate at 20 rpm and incubate overnight (17 hours). Place 400 mL Gene Expression Wash buffer 2 in a sealed bottle and incubate at 37°C overnight along with an empty staining trough.

17. After hybridisation, fill one staining trough with ~400 mL room temperature Agilent Gene Expression Wash buffer 1 (trough 1) and fill a second with Agilent Gene Expression Wash buffer 1 to cover a slide rack (trough 2). Add a slide rack and a stir bar to trough 2.

18. Remove the slide-gasket sandwich from the hybridisation chamber base and submerge in trough 1 without letting go of the slides. Using tweezers, pry the sandwich open from the barcode end keeping the slide numeric barcode facing up. Let the gasket slide drop to the bottom of the staining trough whilst keeping hold of the microarray slide (*see* Note 22).

19. Transfer the microarray slide to the rack in trough 2 (containing Wash buffer 1) and stir using the magnetic plate for 1 minute.

20. Before Wash 1 is complete, fill the pre-heated staining trough (trough 3) with the pre-heated Agilent Gene Expression Wash buffer 2 and add a stir bar.

21. Transfer the slide rack from trough 2 to trough 3 and stir for 1 minute at 37°C (*see* Note 23).

22. Slowly remove the slide rack from Wash buffer 2 minimising droplets forming on the slides. Rest the slide rack on a paper towel.

23. Transfer slides immediately to Agilent slide holders and add ozone-barrier covers (if using Scanners G2565CA or G2565BA). Scan the slides immediately using Agilent Microarray Scanner (G4900DA, G2565CA or G2565BA) at 5 micron resolution. Extract data from image files using Agilent Feature Extraction software.

4 Notes

1: To prepare 500 mL of 5M GTC solution, add guanidine thiocyanate powder to a graduated 500 mL flask. Add approximately 200 mL distilled water, mix and leave in warm room overnight (reaction is endothermic). When GTC powder has dissolved, add remaining constituents, except β-mercaptoethanol. Adjust volume to 500 mL by adding distilled water. Store at room temperature away from direct sunlight. Add β-mercaptoethanol before use. Discard if GTC solution develops a yellow colour.

2: V-bottom universal tubes preferred to Falcon tubes as the bacilli centrifuge into tighter pellets.

3: TRI Reagent® (Sigma-Aldrich) or similar products also acceptable.

4: Plunge sputa into GTC solution within 5 minutes of sampling to retain a representative RNA profile. Mycobacterial transcription ceases on addition of GTC solution and nuclease action is minimised to stabilise the RNA signature. Solutions/centrifugations do not need to be chilled. Mycobacteria should not lyse in the presence of GTC solution however eukaryotic cells and other bacteria may. This serves to reduce background RNA and allows accurate quantification of mycobacteria-derived RNA from sputa; other RNAs will be found in the sputa/GTC supernatant. If the sputa/GTC solution becomes viscous, vortex, syringe or add additional GTC solution to ensure a pellet is able to form during centrifugation.

5: This RNA extraction methodology may be applied to mycobacterial samples from *in vitro* axenic or intracellular infection models. If statistical testing is to be applied to the transcriptional dataset, ensure that appropriate comparator conditions and sample replicates are collected using the same RNA extraction methodology.

6: If performing RNA extraction in batches, which is recommended to ensure consistency, add 1 mL TRIzol to each pellet, transfer to a 2 mL screw-capped tube and store at -70°C. Defrost in batches to resume RNA extraction and RNA amplification.

7: TRIzol effectively sterilises pathogenic mycobacteria, so the rest of this protocol may be conducted outside Category Three Containment conditions - this should be validated according to local biosafety guidelines.

8: It is not necessary to add additional salt to increase precipitation efficiency. For some applications, such as isolating small RNAs, skip this precipitation step and proceed directly to purification using sRNA-compatible columns.

9: A white nucleic acid pellet may be visible but this is not always the case.

10: Elution volumes should be a minimum of 30 μL and a maximum of 100 μL. A second elution is recommended to increase RNA yield.

11: Assess quantity and quality of the RNA immediately (before freezing) or, to avoid freeze-thaw cycles, save 2 μL aliquots of each RNA preparation for Nanodrop and Bioanalyser analysis at a later date.

12: Total RNA input may range from 5 ng – 500 ng. The input RNA for all samples should be equal. Amplification changes the RNA profile, so amplified RNA should never be compared directly to unamplified RNA [12]. All samples to be compared should be amplified together to avoid introducing unnecessary technical variation.

13: Use an incubator for the IVT reaction or a PCR-block with variable heated-lid, so condensation does not build up on the tube lids overnight.

14: Use a ratio of 1 μL ULS-Cy3 per 1 μg aRNA. Prepare the correct number of samples per microarray slide. In this example, an Agilent Technologies SurePrint HD 8x 15k slide, so label samples in batches of 8.

15: The design of the microarray should be taken into consideration when choosing a technique to incorporate Cy3 before hybridisation. In this protocol the ULS labelling system directly labels amplified RNA to hybridise to an 8x15k Agilent Technologies *M.tb* complex microarray slide (ArrayExpress accession number ABUGS-41). To hybridise unamplified RNA to the same array would require conversion to cDNA incorporating Cy3-dCTP [13].

16: The 2x Hybridisation buffer contains surfactant that easily forms bubbles, so vortex mix carefully.

17: To avoid damaging the microarray, maintain a clean work area and handle the slides carefully by the edges, never touching the surfaces. Always wear powder-free gloves.

18: The hybridisation solution is applied onto the gasket slide rather than directly onto the microarray slide, which will be placed onto the gasket slide and samples. The surface tension of the liquid allows the sample to be pipetted into the centre of each well of the gasket without touching the sides. When the microarray is lowered on top, an air bubble forms around the inside edge of the gasket, which serves to mix the sample during hybridisation.

19: Gasket slides come in 4 different formats: 1, 2, 4 and 8x. The hybridisation volumes detailed in this protocol are for use with 8x gasket slides. If using 1, 2 or 4x format, apply 490 μL, 245 μL or 100 μL of the hybridisation solution respectively.

20: Line up the slide between finger and thumb a few mm above and parallel to the gasket, drop into place. The samples should be sandwiched between the gasket and microarray slide. Tap the top of the microarray slide with a pipette tip to ensure slide contact with all the samples. There should be an air pocket surrounding each sample volume within each well of the gasket.

21: Stationary air bubbles will compromise the uniformity of the array hybridisation and may lead to loss of data.

22: Ensure that the array-gasket sandwich stays completely submerged in the wash buffer during disassembly.

23: Wash buffer 2 is a higher stringent buffer than Wash 1, therefore Wash 2 is time sensitive and careful timekeeping is important.

Acknowledgements

LMW was funded by a Brazilian government agency CAPES (Coordination for the Improvement of Higher Education Personnel) PhD visiting fellowship [99999.005648/2014-09]. KAG acknowledges funding from the Wellcome Trust for the Bacterial Microarray Group at St. George's [062511, 080039, and 086547]. SJW was supported by the Wellcome Trust [204538/Z/16/Z] and the PreDiCT-TB consortium (http://www.predict-tb.eu) which is funded from the Innovative Medicines Initiative Joint Undertaking under grant agreement No 115337, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.

References

1. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE III. The transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action. JBiolChem. 2004; 279(38):40174–40184.
2. Waddell SJ, Stabler RA, Laing K, Kremer L, Reynolds RC, Besra GS. The use of microarray analysis to determine the gene expression profiles of Mycobacterium tuberculosis in response to anti-bacterial compounds. Tuberculosis(Edinb). 2004; 84(3–4):263–274. [PubMed: 15207496]
3. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK. Transcriptional Adaptation of Mycobacterium tuberculosis within Macrophages: Insights into the Phagosomal Environment. J Exp Med. 2003; 198(5):693–704. [PubMed: 12953091]
4. Rohde KH, Veiga DF, Caldwell S, Balazsi G, Russell DG. Linking the transcriptional profiles and the physiological states of Mycobacterium tuberculosis during an extended intracellular infection. PLoS Pathog. 2012; 8(6):e1002769.doi: 10.1371/journal.ppat.1002769 [PubMed: 22737072]
5. Tailleux L, Waddell SJ, Pelizzola M, Mortellaro A, Withers M, Tanne A, Castagnoli PR, Gicquel B, Stoker NG, Butcher PD, Foti M, et al. Probing Host Pathogen Cross-Talk by Transcriptional Profiling of Both Mycobacterium tuberculosis and Infected Human Dendritic Cells and Macrophages. PLoSONE. 2008; 3(1):e1403.
6. Talaat AM, Ward SK, Wu CW, Rondon E, Tavano C, Bannantine JP, Lyons R, Johnston SA. Mycobacterial bacilli are metabolically active during chronic tuberculosis in murine lungs: insights from genome-wide transcriptional profiling. J Bacteriol. 2007; 189(11):4265–4274. DOI: 10.1128/JB.00011-07 [PubMed: 17384189]
7. Garton NJ, Waddell SJ, Sherratt AL, Lee SM, Smith RJ, Senner C, Hinds J, Rajakumar K, Adegbola RA, Besra GS, Butcher PD, et al. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. PLoSMed. 2008; 5(4):e75.
8. Rachman H, Strong M, Ulrichs T, Grode L, Schuchhardt J, Mollenkopf H, Kosmiadi GA, Eisenberg D, Kaufmann SH. Unique transcriptome signature of Mycobacterium tuberculosis in pulmonary tuberculosis. InfectImmun. 2006; 74(2):1233–1242.

9. Walter ND, Dolganov GM, Garcia BJ, Worodria W, Andama A, Musisi E, Ayakaka I, Van TT, Voskuil MI, de Jong BC, Davidson RM, et al. Transcriptional Adaptation of Drug-tolerant Mycobacterium tuberculosis During Treatment of Human Tuberculosis. J Infect Dis. 2015; doi: 10.1093/infdis/jiv149

10. Honeyborne I, McHugh TD, Kuittinen I, Cichonska A, Evangelopoulos D, Ronacher K, van Helden PD, Gillespie SH, Fernandez-Reyes D, Walzl G, Rousu J, et al. Profiling persistent tubercule bacilli from patient sputa during therapy predicts early drug efficacy. BMC Med. 2016; 14:68.doi: 10.1186/s12916-016-0609-3 [PubMed: 27055815]

11. Chatterjee A, Saranath D, Bhatter P, Mistry N. Global transcriptional profiling of longitudinal clinical isolates of Mycobacterium tuberculosis exhibiting rapid accumulation of drug resistance. PLoS One. 2013; 8(1):e54717.doi: 10.1371/journal.pone.0054717 [PubMed: 23355892]

12. Waddell SJ, Laing K, Senner C, Butcher PD. Microarray analysis of defined Mycobacterium tuberculosis populations using RNA amplification strategies. BMC Genomics. 2008; 9(1):94. [PubMed: 18298834]

13. Waddell SJ, Butcher PD. Use of DNA arrays to study transcriptional responses to antimycobacterial compounds. Methods Mol Biol. 2010; 642:75–91. DOI: 10.1007/978-1-60327-279-7_6 [PubMed: 20401587]

Europe PMC Funders Author Manuscripts