Supporting Information

Title:

Differential Protein Expression in Exponential and Stationary growth phases of *Mycobacterium avium* subsp. *hominissuis* 104

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*Corresponding authors e-mails: <u>shymaa21@yahoo.com</u> & <u>sohkichi@med.niigata-u.ac.jp</u> ORCID; Enany: 0000-0002-7827-6504 & ORCID; Matsumoto: 0000-0002-6106-7538 **Table S1.** List of primer sequences used in this study for 12 genes that were designed with the publicly available Primer3 software.

Gene name	Primer name	Primer sequences (5'-3')	Product size (bp)
MAV_4093	1 F	CCATCAAGGATCCCGACGAG	135
	1_R	ACTTGAACTGCTCCCAGGTG	
katE	2_F	TCAAGTTCCCCGACTTCGTG	143
	2_R	GTCCGACATCAGCCACATGA	
MAV_0556	3_F	CATCGAGATCCTCAAGGGGC	144
	3_R	ATCAGGTCGATCGCCTTGTC	
MAV_3137	4_F	ACCGGATGCCAAGCTGATC	123
	4_R	AGAATCGCATAGATGGGGGC	
MAV_3806	5_F	CGATCGTGCAGTTGGTGTTC	130
	5_R	TCTTCGACGGCTTGAGTGAC	
apeB	6_F	AAGGGCTCTGCGAGTTCATC	101
	6_R	CTCGTCCAGTTCGGTGTACC	
MAV_1198	7_F	TCAAGAGCGGGTTCTTCGAG	118
	7_R	TTGAGCTCGCGGATCTTCTC	
MAV_3367	8_F	GAACTTCAGCAACGCCAAGG	105
	8_R	GGCATGATCTTGTTCACCGC	
katG	9_F	GGGGTGAAGAGGACGAATGG	129
	9_R	GATCAGACCCATGGTGGTGG	
tuf	10_F	GGTCACGCCGACTACATCAA	150
	10_R	GACCAGGATGTAGGGCACAC	
MAV_4925	11_F	GAAGCTGTTGATGCTCAGCG	146
	11_R	CGTATCCCTCCTTGATCCGC	
MAV_3858	12_F	TCCCCGGTTCGATTTCCTTG	119
	12 R	CCGGACCTCCTTGTTGATCC	



Figure S1: The workflow of our gel spot refining strategy. Three filters (two automated and one manual) were used for refining Progenesis SameSpot selected spots. F1 is a spot volume intensity filter to identify spots different by \geq 2-fold. F2 is statistical ANOVA filter to identify spots significantly different at P \leq 0.05. F3 is manual filtering to eliminate suspected background and noise spots. Visualization was achieved by silver staining before spot picking and was followed by LC-MS/MS analysis of spots.



Figure S2: Gel spot comparison for two representatives differentially expressed proteins between the exponential and the stationary growth phases of MAH 104 using Progenesis SameSpots software; Upper line for spot number 63 (Glucose methanol choline) that is up regulated in the stationary phase and lower line for spot number 17 (Universal stress protein) that is down regulated in the stationary phase. Intensities of the two spots differentially expressed in the exponential and the stationary growth phases were shown in the representative gel image (A). 3D views of the intensities of the targeted spots blotted by SameSpots in both phases are shown (B). The logarithmic normalized volume between the 2 phases was blotted for each spot by SameSpots (C). Spot numbers are corresponding to those listed in Table 2. (D) The standard expression profiles of the twelve differentially expressed proteins between the exponential and the stationary growth phases of MAH 104.



Figure S3: Agarose gel electrophoresis of 12 PCR products of genes responsible for the expression of the 12 differentially expressed proteins between the exponential and the stationary growth phases of *Mycobacteriun avium 104*. Low molecular weight markers are used. Band sizes for each gene product of the 12 genes are summarized in table (1).



Figure S4: *M. avium* RNA in exponential and in stationary growth phases quality on the gel (A), purity and concentrations using eppendorf Bio-spectrometer (B) and integrity; electropherogram profiles for *M. avium* RNA resolved on a Pico Chip of Agilent 2100 Bioanalyzer, Agilent Technologies (C). (D) Gel image formed for *M. avium* RNA using Agilent 2100 Bioanalyzer, Agilent Technologies.



Figure S5: Correlation between the gene expression of each gene and the average normalized volume for each protein of the differentially expressed hits between the two growth phases. Numbers shown here indicating the number of genes and proteins as displayed in table 1.

Red circles; up-regulated, Green circles; down-regulated, Black circles; differ in/between the transcriptomic and the proteomic analysis.