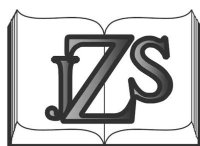

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Part-A- (Pure and Applied Sciences)

High throughput Phenotypic Microarray profiling of *Mycobacterium abscessus*

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Abstract

Mycobacterium abscessus group comprises of five species of rapidly growing mycobacteria. These mycobacteria are increasingly responsible for difficult-to treat, opportunistic cutaneous and respiratory tract infections, notably in cyctic fibrosis patients. Identification at the species level remains problematic and is relying on nucleotide sequence analyses, leaving a need for routine, phenotypic identification of such isolates. Here, the carbon and peptide nitrogen source utilization patterns of *Mycobacterium abscessus* complex mycobacteria was investigated using Biolog phenotype MicroArray analysis based on tetrazolium dye reduction. In a first step, a data base was built after duplicate investigation of *Mycobacterium abscessus, Mycobacterium bolletii*, *Mycobacterium massiliense, Mycobacterium chelonae* and *Mycobacterium franklinii* type strains. In this study, 190 carbon sources were tested; 77 (40.5%), 77 (40.5%), 77 (40.5%), 76 (40%) and 37 (19.5%) were found to be utilized by type strains of *M. abscessus, M. bollettii, M. massiliense, M. chelonae* and *M. franklinii*. Of 285 peptide nitrogen sources, they were found able to utilize 217 (76 %), 216 (75.7 %), 209 (73. 3 %), 194 (68 %) and 24 (8.4 %) nitrogen substrates, respectively. Each species yielded one specific profile, thus allowing for their unambiguous phenotypic identification at the species level. In a second step, a collection of *M. abscessus, M. bolletii, M. massiliense* and *M. chelonae* was tested to assess the usefulness of the Biolog for identification. We observed that all then identical strains were correctly identified at the species level.

Introduction

The *Mycobacterium abscessus* group comprises of difficult to-treat, opportunistic pathogens accountable for clusters of sporadic and of cutaneous and respiratory tract infections [1] and [2]. While the species *M. abscessus* was initially delineated from *Mycobacterium chelonae* using phenotypic traits [3] further *rpoB* sequence-based investigations disclosed *Mycobacterium bolletii* and *Mycobacterium massiliense* as new species in this group [4] and [5]. More recently, *Mycobacterium franklinii* has been described as a fifth member in the *M. abscessus group, after its isolation* in patients with chronic sinusitis in northeastern United States [4]. These five taxons are phenotypically highly similar, using routing tests, being mostly distinguished by DNA sequencing [5] and [6]. This close relatedness even questioned the status of these different taxons with *M. bolletii* and *M. massiliense* being proposed as forming a unique subspecies [7].

We herein used the Phenotype MicroArray (PM Biolog) technology to get insight into the phenotypic diversity of this group of mycobacteria, and to question whether phenotypic profiles could be used for the phenotypic identification of these organisms. The PM is a cellular analysis system that combines proprietary

assays, instrumentation (OmniLog^{TM}) and software in a high-throughput format to study microbial phenotype [8]. This method permits checking of cellular respiration through cell growth on 96-well microplates under a maximum of 1,920 different medium conditions by colorimetrically detection of generation of purple-colored formazane from tetrazolium dye, featuring the intracellular reducing state by NADH [9]. This method has been used to study phenomics of several bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* [12]. As for mycobacteria, the sole published report indicated that 12 substrates differentiated *Mycobacterium bovis* from *Mycobacterium tuberculosis* [10]. Here, we tested whether phenomics using the PM could also discriminate between the closely related *M. abscessus* group mycobacteria, in the perspective of their routine phenotypic identification.

Methods

1. **Mycobacteria.** *M. abscessus, M. bolletii, M. chelonae* and *M. massiliense* clinical isolates (Chest hospital-Sulaimani) and five reference strains *M. abscessus* CIP 104536*, M. bolletii* CIP 108541, *M. massiliense* CIP 108297, *M. chelonae* CIP 104535 and *M. franklinii* DSM 45524 were analysed in this study. Mycobacteria were cultured at 37°C in 7H10 Middlebrook agar supplemented with 10% (v/v) oleic acid/albumin/dextrose/catalase (OADC) (Becton Dickinson, Sparks, MD, USA) and 0.5% (v/v) glycerol for 2-5 days. The identification of all strains was ensured by the matrix-assisted mass spectrometry [11] and partial *rpoB* gene sequencing [5].
2. ***M. abscessus* group phenomics database.** In a first step, we built a phenomics database for one reference strain of each of the 5 *M. abscessus* complex species. Phenotype microarray experiments were conducted based on the standard Biolog Inc. procedure (Biolog, Hayward, USA) as previously reported [8] and [12]. PMs are 96-well microtiter plates which contain well-defined medium incorporating a sole carbon source (PM1 and PM2A Plates) or peptide nitrogen source (PM6, PM7 and PM8 Plates) with the addition of a dye indicator for cell respiration. Colonies were picked from 7H10 medium plates with a cotton swab dipped in 2% Tween 80 and cells were well-suspended in GN/GP-IF-0a (Biolog inoculating 133 fluids) in order to give 81% transmittance using turbidimeter (Biolog). PM additive solutions were made for each plate according to table 1. Each PM plate was inoculated with 100 µL of inoculating fluid consisting of 10 mL IF-0a GN/GP (1.2x), 0.12 mL dye mix H (100x), 1.0 mL PM additive (12x) and mycobacteria in IF-0a GN/GP at (0.88 ml). The PM panels were incubated in the OmniLog instrument (Biolog) for four days at 35˚C for *M. franklinii* and at 37˚C for *M. abscessus, M. bolletii, M. chelonae* and *M. massiliense*. In parallel, the negative control was tested 11 results. Kinetic data were analyzed with OmniLog PM software, V1.3 (Biolog, USA). A clustered heatmap was created using the R statistical software based on the concentration of substrates as a distance matrixed.
3. **Identification of *M. abscessus* complex strains.** In order to assess whether Biolog could be used for the phenotypic identification of *M. abscessus* complex mycobacteria, a collection of clinical isolate was further investigated using plates PM1, PM2A, PM6, PM7 and PM8 the protocol reported above. The obtained patterns were then compared with those enter in the database.
4. **Statistical analysis** Statistical significance for differences between mycobacterial strains for each well calculated by pairwise two-tailed t-test using GraphPad Prism 6 (Software MacKiev, USA) (table 2).

Results

M. abscessus complex phenomics database.

Substrates used in any species, Herein gathered data were interpreted as authentic since the negative controls remained negative in all experiments. Out of the total 190 carbon and 285 peptide nitrogen tested sources (tables 3-8), *M. abscessus, M. bolletii, M. massiliense* and *M. chelonae* were found able to utilize a 77 (40.5%) and 76 (40 %) of carbon substrates and 217 (76 %), 216 (75%), 209 (73%) and 194 (68 %) of nitrogen sources, respectively. Species-specific use substrates, there was no significant difference in the carbon utilization panels between *M. abscessus, M. bolletii* and *M. massiliense,* while PM analysis in the peptide nitrogen panels generated different results in 28 wells which gave a good dye reduction with *M.*

abscessus and *M. bolletii* but were considerably lower with *M. massiliense* and *M. chelonae* strains (figure 1). The carbon and nitrogen catabolism capacity of *M. franklinii* strains tested was extremely low, recording only 37 (19.5 %) of 190 carbon substrates and 24 (8.4 %) of 285 peptide nitrogen substrates tested. For carbon source, M-tartaric acid gave admirable dye reduction with *M. franklinii* (248 Omnilog unit), but were noticeably lower with *M. abscessus, M. chelonae, M. bolletii* and *M. massiliense* strains (71, 53, 130 and 120 Omnilog unit, respectively, $p<0.05$). Sustained dye reduction by five strains of *M. abscessus*, *M. bolletii*, *M. chelonae, M. massiliense* and *M. franklinii* is shown in color scale on the heatmaps (figures 2 and 3). Omnilog unit values are displayed in a heat map, representing higher values in more intense red colors and lower values in more intense orange colors. Basically these data denote dye reduction for 96 h of incubation.

Discussion

Here, we report for the first time the carbon and peptide nitrogen source utilization profile of *M. abscessus* complex mycobacteria using PM. Data were validated by controls and by using a ≥ 5-fold difference in dye reduction as previously proposed for *M. tuberculosis* [10]. Accordingly, substrates commonly used in culture media for mycobacteria yielded significant dye reduction, such as Tween 80 as a carbon source [10]. Growth supplements are widely used to improve the culture yield of mycobacteria, including *M. abscessus* group [13]. For the improved recovery of *M. abscessus*, Middlebrook 7H9 broth (glycerol) has been supplemented with Tween 80 [14]. Also, Tween 80 has been reported to be hydrolyzed by *M. bovis* [10] and [15] and *M. avium* subsp. *paratuberculosis* [16]. Further, *M. smegmatis* grown in the presence of Tween 80 synthesizes series-2 type glycolipids [17]. Likewise, the best nitrogen source across all strains was L-glutamatic acid, the main source for nitrogen in the classic Middlebrook media which are optimal for the culture of the whole Mycobacteria. Using the Biolog OmniLog system, it was observed that *M. abscessus, M. bolletii* and *M. massiliense* used the maximum number of carbon substrates whereas *M. chelonae* and *M. franklinii* used the least. A study provides an insight of rapidly growing Mycobacterium phenotype profiles (*Mycobacterium smegmatis, Mycobacterium fortuitum, Mycobacterium chelonae* and *Mycobacterium phlei*) using Phenotypic MicroArray (PM) system in response to optimal and suboptimal temperature (37ºC and 30ºC), it was observed that *M chelonae* used the minimum number of carbon substrates [19].

The Phenotype MicroArrays yielded a unique phenotypic profile for each one of the five tested species and this profile was constant two strains in the same species allows researchers to distinguish between closely related mycobacteria species. PM analysis in the carbon and peptide nitrogen panels revealed twenty-eight substrates differentiated from species level between *M. abscessus, M. bolletii, M. massiliense, M. chelonae* and *M. franklinii*. These data indicate a phenotypic diversity among *M. abscessus* group mycobacteria and the capacity to discriminate *M. abscessus, M. bolletii, M. chelonae, M. massiliense* and *M. franklinii* on phenotypic bases. These date therefore sustain a previous classification of these five taxons over further proposal to combine *M. bolletii* and *M. massiliense* into an unique taxon; and all as subspecies of an unique; *M. abscessus* species [18]. Using the Biolog Omnilog system, it was observed that *M. abscessus, M. bolletii, M. chelonae* and *M. massiliense* used the greatest number of carbon and nitrogen substrates whereas *M. franklinii* used the least. This study proved that Biolog Phenotype MicroArrays system was able to quickly and reliably identify potential substrates for rapidly growing mycobacteria.

Table- 1: Preparation and concentration of PM additives solutions.

Ingredient	1x Conc.	120x Conc.	Formula Weight	Grams/ 100 ml	PM 1, 2	PM 6, 7, 8
$MgCl_2$, $6H_2O$	2mM	240mM	203.3	4.88	10ml	10ml
$CaCl_2$, $2H_2O$	1mM	120mM	147.0	1.76		
Tween 80	0.01%	1.2%	-	1.2	10ml	10ml
D-glucose	5mM	600mM	180.2	10.8	-	10ml
Middlebrook 7H9 broth (1.2X)	1:100 of 1x	-	-	0.47	10mL	10mL
Sterile water					70ml	60ml
Total					100mL	100mL

Table- 2: Mean, SD, SEM and t-tests data from PM6-PM8.

	Arg-Trp	His-Lys	Lys-Trp	Met-Lys	Phe-Trp	Lys-Met
M. abscessus						
Mean	95.09	94.1	98.449	87.28	63.17	84.72
SD	73.92	71.71	67.13	67.61	44.63	66.62
SEM	3.78	3.66	3.43	3.45	2.28	3.4
M. bolletii						
Mean	85.39	95.74	86.94	98.04	35.64	93.72
SD	67	75.57	71.141	86.22	22.42	73.83
SEM	3.42	3.86	3.64	4.41	1.15	3.77
M. massiliense						
Mean	33.04	43.98	42.36	35.72	28.73	35.83
SD	24.01	27.13	21.85	25.85	18.99	22.36
SEM	1.23	1.39	1.12	1.32	0.97	1.14
M. franklinii						
Mean	10.48	8.08	14.86	6.64	9.08	19.93
SD	3.6	2.86	3.48	2.08	3.15	5.66
SEM	0.18	0.15	0.18	0.11	0.16	0.29
M. chelonae						
Mean	27.64	47.80	44.73	27.45	35.95	16.05
SD	23.73	29.80	52.86	12.47	26.53	7.47
SEM	1.2110	1.5207	2.69750	0.6364	1.3539	0.3812
t-test M. abscessus V M. bolletii	0.0576	0.7574	0.0774	0.0551	0.0001	0.0774
t-test M. abscessus V M. massiliense	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. abscessus V M. franklinii	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. abscessus V M. chelonae	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. chelonae V M. franklinii	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. bolletii V M. massiliense	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. bolletii V M. franklinii	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. bolletii V M. chelonae	0.0001	0.0001	0.0001	0.0001	0.8612	0.0001
t-test M. massiliense V M. franklinii	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
t-test M. massiliense V M. chelonae	0.0018	0.0636	0.4171	0.0001	0.0001	0.0001

Yellow color: significance tests where p<0.05
Green color: not significant p>0.05.

Table- 3: Carbon and peptide nitrogen substrates utilization by *M. abscessus* complex

Organism (NO. of sample)	*Carbone source (PM1 & PM2) n=190 (%)*	*Nitrogen source (PM6,PM7 & PM8) n= 285 (%)*
M. abscessus (2)	*77 (40.5)*	*217 (76)*
M. bolletii (2)	*77 (40.5)*	*216 (75.7)*
M. massiliense (2)	*77 (40.5)*	*209 (73.3)*
M. chelonae (2)	*76 (40)*	*194 (68)*
M. franklinii (1)	*37 (19.5)*	*24 (8.4)*

Table- 4: Carbone sources utilization profile for rapidly growing mycobacteria on PM1 plate.

Well	Substrate	*M. abs.*	*M. boll.*	*M. ch.*	*M. mass.*	*M. fr.*
A2	L-Arabinose	+	+	+	+	+
A3	N-Acetyl-D Glucos amine	+	+	+	+	-
A5	Succinic Acid	+	+	+	+	-
A6	D-Galactose	+	+	+	+	+
A9	D-Alanine	+	+	+	+	-
A10	D-Trehalose	+	+	+	+	-
A11	D-Mannose	+	+	+	+	+
B1	D-Serine	+	+	+	+	+
B3	Glycerol	+	+	+	+	+
B6	D-Gluconic Acid	+	+	+	+	-
B8	D-Xylose	+	+	+	+	+
B10	Formic Acid	+	+	+	+	-
B12	L-Glutamic Acid	+	+	+	+	+
C3	D,L-Malic Acid	+	+	+	+	-
C4	D-Ribose	+	+	+	+	+
C5	Tween 20	+	+	+	+	+
C8	Acetic Acid	+	+	+	+	-
C9	α-D-Glucose	+	+	+	+	-
C12	Thymidine	+	+	+	+	-
D1	L-Asparagine	+	+	+	+	+
D5	Tween 40	+	+	+	+	-
D7	α-Keto -Butyric Acid	+	+	+	+	-
E1	L-Glutamine	+	+	+	+	-
E2	M-Tartaric Acid	-	-	-	-	+
E4	D-Fructose-6-Phosphate	+	+	+	+	+
E5	Tween 80	+	+	+	+	+
E7	α-Hydroxy Butyric Acid	+	+	+	+	-
F1	Glycyl-L-Aspartic Acid	+	+	+	+	+
F2	Citric Acid	+	+	+	+	-
F4	D-Threonine	+	+	-	+	-
F5	Fumaric Acid	+	+	+	+	-
F7	Propionic Acid	+	+	+	+	-

Well	Substrate	*M. abs.*	*M. boll.*	*M. ch.*	*M. mass.*	*M. fr.*
F10	Glyoxylic Acid	+	+	+	+	-
G1	Glycyl -L -Glutamic Acid	+	+	+	+	+
G3	L-Serine	+	+	+	+	-
G5	L-Alanine	+	+	+	+	-
G6	L-Alanyl -Glycine	+	+	+	+	-
G7	Acetoacetic Acid	+	+	+	+	+
G9	Mono Methyl Succinate	+	+	+	+	-
G10	Methyl Pyruvate	+	+	+	+	+
G12	L-Malic Acid	+	+	+	+	-
H1	Glycyl -L -proline	+	+	+	+	-
H5	D-Psicose	+	+	+	+	+
H6	L-Lyxose	+	+	+	+	+
H7	Glucuron amide	+	+	+	+	+
H8	Pyruvic Acid	+	+	+	+	+
H10	D-Galacturonic Acid	+	+	+	+	+
H12	2-Aminoethanol	+	+	+	+	-

M. abs= M. abscessus; M. boll.= M. bolletii; M. mass.= M. massiliense; M. fr.= M. franklinii ; M. ch.= M. chelonae
.

Table- 5: Carbone sources utilization profile for rapidly growing mycobacteria on PM2 plate.

well	Substrate	M. abs.	M. boll.	M. ch.	M. mass.	M. fr.
A10	Laminarin	+	+	+	+	+
A11	Mannan	+	+	+	+	+
A12	Pectin	+	+	+	+	+
B5	D-Arabinose	+	+	+	+	+
B8	Arbutin	+	+	+	+	-
B9	2-Deoxy-D-Ribose	+	+	+	+	+
B12	3-0 -β -Dgalacto-pyranosy l-Darabinose	+	+	+	+	+
C12	Palatinose	+	+	+	+	+
D6	D-Tagatose	+	+	+	+	+
D9	N-acetyle L-glutamic acid	+	+	+	+	-
D10	γ –Amino Butyric Acid	+	+	+	+	-
D12	Butyric Acid	+	+	+	+	+
E2	Caproic Acid	+	+	+	+	-
E5	D-Glucosamine	+	+	+	+	+
E8	β-Hydroxy Butyric Acid	+	+	+	+	-
E10	α-Keto Valeric Acid	+	+	+	+	-
E12	5-Keto-D-Gluconic Acid	+	+	+	+	+
F5	Oxalomalic Acid	+	+	+	+	+
F8	Sebacic Acid	+	+	+	+	-
F9	Sorbic Acid	+	+	+	+	+
G4	L-Arginine	+	+	+	+	-
G9	L- Isoleucine	+	+	+	+	-
G10	L-Leucine	+	+	+	+	-
G11	L-Lysine	+	+	+	+	-
G12	L-Methionine	+	+	+	+	-
H1	L-Ornithine	+	+	+	+	-
H8	Putrescine	+	+	+	+	-
H9	Dihydroxy acetone	+	+	+	+	+
H10	2,3 -Butanediol	+	+	+	+	-
H12	3-Hydroxy 2-Butanone	+	+	+	+	+

Table- 6: Nitrogen sources utilization profile for rapidly growing mycobacteria on PM6 plate.

Well	Substrate	M. abs.	M. boll.	M. ch.	M. mass.	M. fr.
A2	L-Glutamine	+	+	+	+	-
A3	Ala-Ala	+	+	+	+	-
A4	Ala-Arg	+	+	+	+	-
A5	Ala-Asn	+	+	+	+	-
A6	Ala-Glu	+	+	+	+	-
A7	Ala-Gly	+	+	+	+	-
A8	Ala-His	+	+	+	+	-
A9	Ala-Leu	+	+	+	+	-
A10	Ala-Lys	+	+	+	+	+
A11	Ala-Phe	+	+	+	+	-
A12	Ala -Pro	+	+	+	+	-
B1	Ala-Ser	+	+	+	+	-
B03	Ala-Trp	+	+	+	+	-
B04	Ala-Tyr	+	+	+	+	-
B05	Arg-Ala	+	+	+	+	-
06	Arg-Arg	+	+	+	+	-
B07	Arg-Asp	+	+	+	+	-
B08	Arg-Gln	+	+	+	+	-
B09	Arg-Glu	+	+	+	+	-
B10	Arg-Ile	+	+	+	+	-
B11	Arg-Leu	+	+	+	+	-
B12	Arg-Lys	+	+	+	+	+
C01	Arg-Met	+	+	-	+	-
C02	Arg-Phe	+	+	+	+	-
C03	Arg-Ser	+	+	+	+	-
C04	Arg-Trp	+	+	+	-	-
C05	Arg-Tyr	+	+	+	+	-
C06	Arg-Val	+	+	-	+	+
C07	Asn-Glu	+	+	+	+	-
C08	Asn-Val	+	+	+	+	-
C10	Asp-Glu	+	+	+	+	
C11	Asp-Leu	+	+	+	+	-
C12	Asp-Lys	+	+	+	+	-
D01	Asp-Phe	+	+	+	+	-
D03	Asp-Val	+	+	+	+	-
D05	Gln-Gln	+	+	+	+	-
D06	Gln-Gly	+	+	+	+	-
D08	Glu-Glu	+	+	+	+	+
D09	Glu-Gly	+	+	+	+	-
D10	Glu-Ser	+	+	+	+	+
D12	Glu-Tyr	+	+	+	+	
E01	Glu-Val	+	+	+	+	-
E02	Gly-Ala	+	+	+	+	-
E03	Gly-Arg	+	+	+	+	-
E06	Gly-His	+	+	+	+	-
E07	Gly-Leu	+	+	+	+	-
E08	Gly-Lys	+	+	+	+	-
E09	Gly-Met	+	+	-	+	-
E10	Gly-Phe	+	+	+	+	-

Well	Substrate	M. abs.	M. boll.	M. ch	M. mass.	M. fr.
E11	Gly-Pro	+	+	+	+	-
E12	Gly-Ser	+	+	+	+	-
F03	Gly-Tyr	+	+	+	+	-
F04	Gly-Val	+	+	+	+	-
F07	His-Leu	+	+	+	+	-
F08	His-Lys	+	+	+	-	-
F11	His -Se r	+	+	+	+	-
G03	Ile-Ala	+	+	+	+	-
G04	Ile-Arg	+	+	+	+	-
G05	Ile-Gln	+	+	+	+	-
G06	Ile-Gly	+	+	+	+	-
G07	Ile-His	+	+	+	+	-
G08	Ile-Ile	+	+	+	+	-
G10	Ile-Phe	+	+	+	+	-
G11	Ile-Pro	+	+	+	+	-
G12	Ile-Ser	+	+	+	+	-
H03	Ile-Val	+	+	+	+	-
H04	Leu-Ala	+	+	+	+	-
H05	Leu-Arg	+	+	+	+	-
H06	Leu-Asp	+	+	+	+	-
H07	Leu-Glu	+	+	+	+	-
H08	Leu-Gly	+	+	+	+	-
H09	Leu-Ile	+	+	+	+	-
H10	Leu-Leu	+	+	+	+	-
H11	Leu-Met	+	+	+	+	-

Table- 7: Nitrogen sources utilization profile for rapidly growing mycobacteria on PM7

Well	*Substrate*	*M. abs.*	*M. boll.*	*M.ch*	*M. mass.*	*M. fr.*
A02	*L-Glutamine*	+	+	+	+	-
A03	*Leu-Ser*	+	+	+	+	-
A04	*Leu-Trp*	+	+	+	+	-
A05	*Leu-Val*	+	+	+	+	-
A06	*Lys-Ala*	+	+	+	+	+
A07	*Lys-Arg*	+	+	+	+	-
A08	*Lys-Glu*	+	+	+	+	+
A09	*Lys-Ile*	+	+	+	+	-
A10	*Lys-Leu*	+	+	+	+	+
A11	*Lys-Lys*	+	+	+	+	+
A12	*Lys-Phe*	+	+	-	+	-
B01	*Lys-Pro*	+	+	+	+	-
B02	*Lys-Ser*	+	+	+	+	-
B04	*Lys-Trp*	+	+	+	-	-
B05	*Lys-Tyr*	+	+	+	+	-
B06	*Lys-Val*	+	+	+	+	-
B07	*Met-Arg*	+	+	-	+	-
B08	*Met-Asp*	+	+	-	+	-
B09	*Met-Gln*	+	+	-	+	-
B10	*Met-Glu*	+	+	-	+	+
B11	*Met-Gly*	+	+	-	+	-
B12	*Met-His*	+	+	-	+	+
C01	*Met-Ile*	+	+	+	+	-
C02	*Met-Leu*	+	+	-	+	-
C03	*Met-Lys*	+	+	-	-	-
C06	*Met-Pro*	+	+	-	+	-
C08	*Met-Val*	+	+	-	+	-
C09	*Phe-Ala*	+	+	+	+	-
C10	*Phe-Gly*	+	+	+	+	-
C11	*Phe-Ile*	+	+	+	+	-
C12	*Phe-Phe*	+	+	+	+	+
D02	*Phe-Ser*	+	+	+	+	-
D03	*Phe-Trp*	+	-	-	-	-
D04	*Pro-Ala*	+	+	+	+	-
D05	*Pro-Asp*	+	+	+	+	-
D06	*Pro-Gln*	+	+	+	+	-
D07	*Pro-Gly*	+	+	+	+	-
D09	*Pro-Leu*	+	+	+	+	-
D10	*Pro-Phe*	+	+	+	+	-
D11	*Pro-Pro*	+	+	+	+	-
D12	*Pro-Tyr*	+	+	+	+	-
E01	*Ser-Ala*	+	+	+	+	+
E02	*Ser-Gly*	+	+	+	+	+
E03	*Ser-His*	+	+	+	+	+

Well	*Substrate*	*M. abs.*	*M. boll.*	*M. ch.*	*M. mass.*	*M. fr.*
E04	*Ser-Leu*	+	+	+	+	+
E05	*Ser-Met*	+	+	-	+	-
E06	*Ser-Phe*	+	+	+	+	-
E07	*Ser-Pro*	+	+	+	+	-
E08	*Ser-Ser*	+	+	+	+	-
E09	*Ser-Tyr*	+	+	+	+	-
E10	*Ser-Val*	+	+	+	+	+
F4	*Thr-Met*	+	+	+	-	+
F06	*Trp-Ala*	+	+	+	+	-
F07	*Trp-Arg*	+	+	-	+	-
F08	*Trp-Asp*	+	+	+	+	-
F09	*Trp-Glu*	+	+	+	+	-
F10	*Trp-Gly*	+	+	+	+	+
F11	*Trp-Leu*	+	+	+	+	-
F12	*Trp-Lys*	+	+	+	+	+
G02	*Trp-Ser*	+	+	+	+	-
G03	*Trp-Trp*	+	+	+	+	-
G05	*Tyr-Ala*	+	+	+	+	-
G06	*Tyr-Gln*	+	+	-	+	-
G07	*Tyr-Glu*	+	+	+	+	-
G08	*Tyr-Gly*	+	+	+	+	-
G10	*Tyr-Leu*	+	+	+	+	-
G11	*Tyr-Lys*	+	+	+	+	-
H02	*Tyr-Tyr*	+	+	+	-	+
H03	*Val-Arg*	+	+	+	+	-
H04	*Val-Asn*	+	+	+	+	-
H05	*Val-Asp*	+	+	+	+	+
H06	*Val-Gly*	+	+	+	+	-
H07	*Val-His*	+	+	+	+	-
H08	*Val-Ile*	+	+	+	+	-
H09	*Val-Leu*	+	+	+	+	-
H10	*Val-Tyr*	+	+	+	+	-
H11	*Val-Val*	+	+	+	+	-

Table- 8: Nitrogen sources utilization profile for rapidly growing mycobacteria on PM8 plate.

Well	Substrate	M. abs.	M. boll.	M. ch	M. mass.	M. fr.
A02	L-Glutamine	+	+	+	+	-
A03	Ala-Asp	+	+	+	+	-
A04	Ala-Gln	+	+	+	+	-
A05	Ala-Ile	+	+	+	+	-
A06	Ala-Met	+	+	+	+	-
A07	Ala-Val	+	+	+	+	-
A08	Asp-Ala	+	+	+	+	-
A09	Asp-Gln	+	+	+	+	-
A10	Asp-Gly	+	+	+	+	-
A11	Glu-Ala	+	+	+	+	-
A12	Gly-Asn	+	+	+	+	-
B01	Gly-Asp	+	+	+	+	-
B02	Gly-Ile	+	+	+	+	-
B03	His-Ala	+	+	+	+	-
B04	His-Glu	+	+	+	+	-
B06	Ile-Asn	+	+	+	+	-
B07	Ile-Leu	+	+	+	+	-
B08	Leu-Asn	+	+	+	+	-
B09	Leu-His	+	+	+	+	-
B10	Leu-Pro	+	+	+	+	-
B11	Leu-Tyr	+	+	+	+	-
B12	Lys-Asp	+	+	+	+	-
C01	Lys-Gly	+	+	+	+	+
C02	Lys-Met	+	+	-	-	-
C03	Met-Thr	+	+	-	+	-
C05	Phe-Asp	+	+	+	+	-
C06	Phe-Glu	+	+	+	+	-
C07	Gln-Glu	+	+	-	+	-
C08	Phe-Met	+	+	+	+	-
C09	Phe-Tyr	+	+	+	+	-
C10	Phe-Val	+	+	+	+	-
C11	Pro-Arg	+	+	+	+	-
C12	Pro-Asn	+	+	+	+	-
D01	Pro-Glu	+	+	+	+	-
D02	Pro-lle	+	+	-	+	-
D03	Pro-Lys	+	+	-	+	-
D04	Pro-Ser	+	+	+	+	-
D06	Pro-Val	+	+	+	+	-
D07	Ser-Asn	+	+	+	+	+
D08	Ser-Asp	+	+	+	+	-

Well	Substrate	M. abs.	M. boll.	M. ch	M. mass.	M. fr.
D09	Ser-Gln	+	+	+	+	-
D10	Ser-Glu	+	+	+	+	-
E06	Val-Ala	+	+	+	+	-
E07	Val-Gln	+	+	+	+	-
E08	Val-Glu	+	+	+	+	-
E09	Val-Lys	+	+	+	+	-
E10	Val-Met	+	+	+	+	-
E11	Val-Phe	+	+	+	+	-
E12	Val-Pro	+	+	+	+	-
F01	Val-Ser	+	+	+	+	-
F12	D-Leu-Tyr	+	+	+	+	-
G01	g-Glu-Gly	+	+	+	+	-
G08	Leu-b-Ala	+	+	+	+	-
G10	Phe-b-Ala	+	+	+	+	-
G11	Ala-Ala-Ala	+	+	+	+	-
G12	D-Ala-Gly-Gly	+	+	+	+	-
H01	Gly-Gly-Ala	+	+	+	+	-
H02	Gly-Gly-D-Leu	+	+	+	+	-
H04	Gly-Gly-Ile	+	+	+	+	-
H05	Gly-Gly-Leu	+	+	+	+	-
H06	Gly-Gly-Phe	+	+	+	+	-
H08	Gly-Phe-Phe	+	+	+	+	-
H09	Leu-Gly-Gly	+	+	+	+	-
H10	Leu-Leu-Leu	+	+	+	+	-
H11	Phe-Gly-Gly	+	+	+	+	-
H12	Tyr-Gly-Gly	+	+	+	+	-

Figure- 1: Phenotype array results for peptide nitrogen sources comparing between five rapidly growing mycobacteria.

Figure- 2: Phenotype array results for carbon sources (PM1 and PM2A), row represents *M. abscessus* group and column represents carbon source (Incubation period = 96 hour).

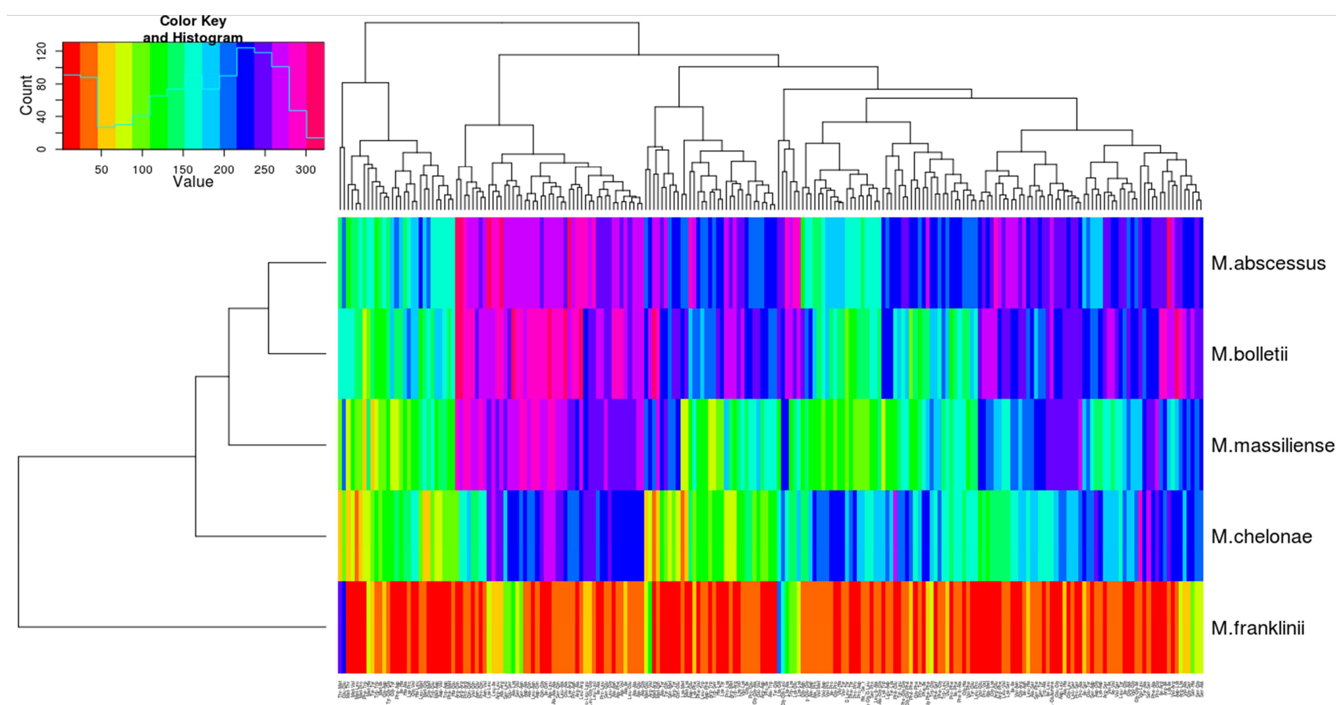

Figure- 3: Phenotype array results for peptide nitrogen sources (PM6, PM7 and PM2A), row represents *M. abscessus* group and column represents peptide nitrogen source (Incubation period = 96 hour).

References

[1] Sassi, M. Ben Kahla, I. Drancourt, M . *"Mycobacterium abscessus multispacer sequence typing"*. BMC Microbiol. Vol.13, pp.3. (2013).

[2] Furuya, E.Y. Paez, A. Srinivasan, A. Cooksey, R. Augenbraun, M. et al. *"Outbreak of Mycobacterium abscessus wound infections among "lipotourists" from the United States who underwent abdominoplasty in the Dominican Republic"*. Clin Infect Dis. Vol .46**,** pp.1181-1188. (2008).

[3] Kusunoki, S. Ezaki,T. *"Proposal of Mycobacterium peregrinum* sp. nov., nom.rev. and elevation of *Mycobacterium chelonae* subsp. abscessus (Kubica et al.) *to species status:Mycobacterium abscessus* comb. nov". Int J Syst Bacteriol. Vol. 42, pp. 240-245. (1992).

[4] Simmon, K.E., Brown-Elliott, B.A., Ridge, P.G. Durtschi, J.D. Mann, L.B.et al. "*Mycobacterium chelonae*-abscessus *complex associated with sinopulmonary disease, Northeastern USA"*. Emerg Infect Dis. Vol. 17, pp.1692-1700. (2011).

[5] Adekambi, T.Berger, P. Raoult, D. Drancourt, M. *"rpoB gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of Mycobacterium bolletii sp. nov., Mycobacterium phocaicum sp. nov. and Mycobacterium aubagnense sp. Nov"*. Int J Syst Evol Microbiol . Vol. 56, pp.133-143. (2006).

[6] Adékambi, T. Reynaud-Gaubert, M. Greub, G. Gevaudan, M.J. La Scola B, et al. *"Amoebal coculture of "Mycobacterium massiliense" sp. nov. from the sputum of a patient with hemoptoic pneumonia"*. J Clin Microbiol. Vol .42, 5493-5501. (2004).

[7] Macheras, E. Roux AL, Bastian, S. Leão, S.C. Palaci, M. et al. *"Multilocus sequence analysis and rpoB sequencing of Mycobacterium abscessus (sensu lato) strain*s*"*. J Clin Microbiol. Vol .49, 491-499. (2011).

[8] Bochner, B. R. *"Global phenotypic characterization of bacteria"*. FEMS Microbiol Rev. Vol**.** 33,191-205. (2009).

[9] Bochner, B. R. *"New technologies to assess genotype-phenotype relationships"*. Nat Rev Genet. Vol. 4, pp. 309-314. (2003).

[10] Khatri, B. Fielder, M. Jones, G. Newell, W. Abu-Oun, M. et al. *"High Throughput Phenotypic Analysis of Mycobacterium tuberculosis and Mycobacterium bovis Strains' Metabolism Using Biolog Phenotype Microarrays"*. PLoS One. Vol. 8, pp.e52673. (2013).

[11] El Khéchine, A. Couderc, C. Flaudrops, C. Raoult, D. Drancourt, M. *"Matrix-assisted laser desorption/i,onization time-of-flight mass spectrometry identification of mycobacteria in routine clinical practice"*. PLoS One. Vol. 6, pp. e24720. (2011).

[12] Bochner, B. R. Gadzinski, P. and Panomitros, E. *"Phenotype microarrays for high-throughput phenotypic testing and assay of gene function"*. Genome Res. Vol. 11, pp.1246-1255. (2001).

[13] Brittle, W. Marais, B.J. Hesseling, A.C. Schaaf, H.S. Kidd, M, et al. "*Improvement in mycobacterial yield and reduced time to detection in pediatric samples by use of a nutrient broth growth supplement*". J Clin Microbiol. Vol. 47, pp.1287-1289. (2009).

[14] Pawlik, A. Garnier, G. Orgeur, M. Tong, P. Lohan, A. et al. *"Identification and characterization of the genetic changes responsible for the characteristic smooth-to-rough morphotype alterations of clinically persistent Mycobacterium abscessus"*. Mol Microbiol. Vol. 90, pp.612-29. (2013).

[15] Hayashi, D. Takii, T. Mukai, T. Makino, M. Yasuda, E. et al. "*Biochemical characteristics among Mycobacterium bovis BCG substrains"*. FEMS Microbiol Lett. Vol. 306, pp.103-109. (2010).

[16] Chen, J.W. Scaria, J. Chang, Y.F. *"Phenotypic and transcriptomic response of auxotrophic Mycobacterium avium subsp. paratuberculosis leuD mutant under environmental stress"*. PLoS One. Vol. **7**, pp. e37884. (2012).

[17] Wang, C. Mahrous, E.A. Lee, R.E.Vestling, M.M. Takayama, K. *"Novel Polyoxyethylene-Containing Glycolipids Are Synthesized in Corynebacterium matruchotii and Mycobacterium smegmatis Cultured in the Presence of Tween 80"*. J Lipids: 676535. (2011).

[18] Leao, S.C. Tortoli, E. Euzéby, J.P. Garcia, M.J. *"Proposal that Mycobacterium massiliense and Mycobacterium bolletii be united and reclassified as Mycobacterium abscessus subsp. bolletii comb. nov., designation of Mycobacterium abscessus subsp. abscessus subsp. nov. and emended description of Mycobacterium abscessus"*. Int J Syst Evol Microbiol. Vol. 61, pp.2311–2313. (2011).

[19] Rahman, M. Jaques, III S. Daniels, L. *"Carbon source utilization pattern among rapidly growing Mycobacterium (RGM) species by Phenotypic Array analysis using Biolog OmniLog System"*. SC-ASM Conference, Austin. (2008).