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Proteomics and mass spectrometric studies reveal planktonic growth of *Mycobacterium smegmatis* in biofilm cultures in the absence of *rpoZ*☆

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Abstract

Mycobacterium smegmatis is known to form biofilms and many cell surface molecules like core glycopeptidolipids and short-chain mycolates appear to play important role in the process. However, the involvement of the cell surface molecules in mycobacteria towards complete maturation of biofilms is still not clear. This work demonstrates the importance of the glycopeptidolipid species with hydroxylated alkyl chain and the epoxylated mycolic acids, during the process of biofilm development. In our previous study, we reported the impairment of biofilm formation in *rpoZ*-deleted *M. smegmatis*, where *rpoZ* codes for the ω subunit of RNA polymerase (R. Mathew, R. Mukherjee, R. Balachandar, D. Chatterji, Microbiology 152 (2006) 1741). Here we report the occurrence of planktonic growth in a mc^2155 strain which is devoid of *rpoZ* gene. This strain is deficient in selective incorporation of the hydroxylated glycopeptidolipids and the epoxy mycolates to their respective locations in the cell wall. Hence it forms a mutant biofilm defective in maturation, wherein the cells undertake various alternative metabolic pathways to survive in an environment where oxygen, the terminal electron acceptor, is limiting.

Keywords: Mycobacteria; Biofilm; Proteomics; Mycolates; Glycopeptidolipids

1. Introduction

After a period of extensive work on pure planktonic cultures, an increasing attention has shifted in studying microorganisms in their natural environment, where they are found growing as surface-adherent communities. Biofilms are surface-bound communities of multicellular aggregates where individual microorganisms are held together by an extracellular matrix [1]. This particular behavior of occurrence in multicellularity while maintaining their unicellular existence has attracted much research [2]. The primary advantage of growing in a biofilm is the protection from environmental assaults and survival under low nutrient availability. Moreover, it also increases the ability of the microorganism to evade the action of various antibiotics and disinfectants, thus having direct implication on persistence and virulence [3–6]. Among the bacterial pathogens, biofilm formation has been detected in *Pseudomonas*, *Haemophilus*, *Escherichia*, *Vibrio*, *Salmonella*, *Streptococcus*, *Klebsiella*, *Staphylococcus* and *Mycobacterium* genera, though its presence in *M. tuberculosis* and *M. leprae* is still not clearly shown. However, several non-tuberculous free-living mycobacteria, of which some are opportunistic pathogens, are known to grow as a biofilm. *M. ulcerans* and *M. cheloane*, causative agents of acute skin ulcer, do also exist in a biofilm in the aquatic environment [7–9]. Members of MAC, *M. avium* and *M. xenopi* biofilms are detected in public water distribution systems and dental units [10,11]. *M. fortuitum* and *M. marinum* are among the other fast growing mycobacterium known to form stable biofilms [12,13]. The saprophyte *M. smegmatis*, first isolated from a biofilm in a mammalian smegma, has been well established to grow as a biofilm [14–16].

Formation of biofilms is mainly due to certain characteristic properties of the cell wall and the capsules which can be attributed to the cell–cell adhesion through an extracellular matrix. The shift from planktonic to biofilm mode of growth

Abbreviations: IPG, Immobilized pH gradient; MALDI-TOF, Matrix-assisted laser desorption ionization time of flight

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involves various complex transitions in the gene expression pattern, resulting in a switch over to a production of various structurally diverse metabolites which decorate the outer surface [17]. Decades of targeted mutagenesis studies have resulted in identification of various biosynthetic enzymes and dedicated chaperons which stabilize other enzymes that synthesize, transport or modify the cell surface building blocks. *M. smegmatis* mutants lacking either the undecaprenyl phosphokinase, glycopeptidolipid (GPL) synthetic genes and mycolic acid synthetic enzymes were defective in biofilm formation, which was a direct implication of the role of cell wall lipids in adherence and pellicle formation [15,16,18]. Apart from the importance of GPLs in biofilm, Ojha et al. recently proposed the role of short-chain mycolates for biofilm initiation and its transition to maturation [16]. Necessity for a new mycolate-derived hydrophobic metabolite mycolyl-diacylglycerols has also been discovered [19]. However, the well documented GPLs and mycolates have never been analyzed from a mature biofilm culture. It has also been reported that the biofilm-forming ability gets abrogated due to a defect in a putative DNA-binding transcription factor [19]. The expression profile of various genes and gene products in a sessile growth has been found to be distinct, with its unique manifestations displayed through the adherent bacterial phenotypes [20–22]. Overexpression of various general stress and stationary-phase genes is required for a biofilm growth, thus implicating the altered constitution of the bacterial RNA polymerase with different stress-specific sigma factors [23,24]. Proteomic analysis has also revealed changes in phenotype during biofilm maturations, proteins involved in motility and stress has been cited to be important for biofilm initiation in few organisms [25,26], thus it is intriguing to study the differential protein expression in this non-flagellated mycobacterium having the ability to slide on an agar surface [27].

Bacterial core RNA polymerase is an assembly of two α subunits, one each of β and β′ and ω subunits [28]. ω, the smallest subunit, has been shown to be an integral part of the assembly which helps to fold the β′ subunit and protect it physically [29,30]. We have reported earlier that the *rpoZ*-deleted mc^{2}155 strain (mcdrz) displays various pleiotropic phenotypes like altered colony morphology, minimal sliding motility, defective biofilm formation and inability to produce extracellular matrix [30,31]. However, all surface-related phenomena were observed without any alteration of the cell surface composition in planktonic cultures; therefore it was very interesting to elucidate the molecular mechanism involved in this deficiency. We here report the occurrence of an altered profile of GPLs and mycolates in mc^{2}155 biofilm cultures and try to address the defective biofilm mode of growth in an *rpoZ*-deleted strain at the molecular level, using the tool of proteomics and tandem mass spectrometry.

2. Experimental

2.1. Bacterial strains and growth conditions

M. smegmatis was grown in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween-80 (Sigma) and 2% glucose for planktonic cultures. For biofilm formation *M. smegmatis* was grown in Sauton's medium (Himedia) with 2% glucose in the absence of any surfactants and incubated in a humidified incubator without shaking. Kanamycin (30 μg kanamycin ml^{-1}) was used for the mcdrz strain except for the biofilm cultures [31]. For harvesting cells from biofilm cultures, the floating cells were scraped out and stirred for few hours at 4 °C, in the presence of 0.05% Tween-80 following centrifugation at 4000 rpm for 15 min.

2.2. Purification and analysis of cell wall lipids

The GPLs from *M. smegmatis* mc^{2}155 and mcdrz cells were purified as mentioned earlier [32]. Lipids were extracted with $CHCl_3/CH_3OH$ (2:1) at room temperature for 24 h. The organic supernatant was dried and dissolved in $CHCl_3/CH_3OH$ (2:1) and deacylated by treating with equal volume of 0.2 M NaOH in CH_3OH at 37 °C for 30 min, then neutralized with a few drops of glacial acetic acid. After drying the solvents, lipids were dissolved in $CHCl_3/CH_3OH/H_2O$ (4:2:1) and centrifuged. The aqueous layer was discarded and the organic layer containing the lipids was washed with super-saturated brine and concentrated. The deacylated lipids were spotted on to silica coated TLC plates (Merck), and developed with $CHCl_3/CH_3OH$ (9:1). The sugar-containing lipids were visualized by spraying 10% H_2SO_4 in ethanol, followed by charring the separated spots at 120 °C for 10 min.

For mycolic acid analysis biofilms were harvested from mc^{2}155 and mcdrz cultures grown for the required period of time in Petri dishes as mentioned already. The low amount of biofilms formed by mcdrz necessitated harvesting from 20 plates to obtain adequate cell mass equal to mc^{2}155. The harvested films were refluxed overnight with 50 ml of tetrabutylammonium hydroxide (15%) at 100 °C, followed by addition of 50 ml of dichloromethane and 2.5 ml of methyl iodide, and stirred at room temperature for 1 h. After centrifugation at 10,000 rpm for 10 min, the organic phase was separated and washed once with 0.2N HCl and once with water, then dried under vacuum. The residue was resuspended in 5 ml of dichloromethane and spotted on a TLC plate and developed thrice in petroleum ether:ethyl acetate (95:5, v/v) [33]. The TLC plates were visualized as mentioned above.

2.3. Mass spectrometry

Each GPL species was eluted from preparative TLC silica plates (20 cm × 20 cm) by dissolving in $CHCl_3/CH_3OH$ (2:1). The total mycolates, GPLs and the purified species were mixed with equal volume of matrix solution (dihydroxy benzoic acid in ethanol), and then allowed to co-crystallize at room temperature. MALDI-TOF-MS and MS-MS spectra were acquired on a Bruker Daltonics, ULTRA FLEX TOF-TOF instrument equipped with a pulsed N_2 laser and analyzed in the reflectron mode using a time delay of 90 ns, accelerating voltage of 25 kV in the positive ion mode. To improve the signal-to-noise ratio an average of 500 shots were taken for each spectrum. External calibration to a spectrum, acquired for a mixture of peptides

with masses ranging from 1046 to 2465 Da, was done prior to acquisition.

2.4. Protein extraction and 2D PAGE

Biofilm cultures grown till 7 days were harvested by centrifugation. Bacterial pellets thus obtained were washed once with 10% (w/v) sucrose and dissolved in a lysis buffer containing 50 mM NaCl, 5% (v/v) β-mercaptoethanol, 1% (v/v) IPG buffer (Amersham Biosciences), 6% (v/v) Triton-X 100 and protease inhibitor cocktails (Roche) to a final concentration of 10 mg ml^{-1}. Cells were disrupted by sonication (Sonics Vibra Cell); thereafter the lysate was precipitated, reconstituted in a rehydration solution and loaded on pH 4–7 linear, 18 cm Immobiline dry strips (Amersham Biosciences). The IPG strips were actively rehydrated with 900 μg of protein and focused on an Ettan IPGphor II electrophoresis unit (Amersham Biosciences) as mentioned earlier [34]. The focused strips were equilibrated in SDS buffer with DTT and iodoacetamide for reduction and alkylation successively. Second dimension was run on vertical 7.5 and 15% acrylamide SDS–PAGE in a Hoeffer SE-600 unit; thereafter gels were visualized by staining with silver. The digitized images of wet gels were imported on to Ettan Progenesis software (Amersham Biosciences) and spots were detected from each of the gels against the background. Consequently, the spots in each gel were matched by overlaying to the reference gel.

2.5. Protein identification by MALDI-TOF-MS-MS

Protein spots of interest were excised into 1 mm × 1 mm pieces, then destained with 15 mM K_3FeCN_6 and 50 mM $Na_2S_2O_3$ for 10 min, thereafter with 50 mM NH_4HCO_3 in 50% (v/v) acetonitrile twice for 15 min. The destained gel pieces were dehydrated in acetonitrile for 5 min, dried and reswelled in 50 mM NH_4HCO_3 containing 0.02 μg μl^{-1} sequencing-grade modified trypsin (Promega). The swelled gel pieces were incubated at 37 °C overnight. After digestion the extracted peptides were analyzed by MALDI-TOF-MS as reported earlier [34] and the most intense peaks in the spectrum were selected for fragmentation by laser-induced dissociation (LID). For tandem mass spectrometry an average of 1000 laser shots were accumulated and the spectrum was calibrated internally to the precursor ion mass. These MS-MS spectra were used for sequence specific search at the Mascot database (Matrix Science, UK).

3. Results and discussion

3.1. Altered profile of the cell surface glycopeptidolipids

Surface hydrophobicity is a major contributor to cell–cell adhesion and that to the polyvinyl chloride surfaces. GPLs are among the non-covalently bound components of non-tuberculous mycobacterial outer surface [35]. These amphiphilic molecules with a long alkyl chain and a polar head, consisting of deoxy sugars attached to the peptidolipid core [36], have been extensively associated with biofilm initiation process and sliding motility [15,37]. Even the GPLs defective in their acetylation at the 6-deoxytalose moiety have been shown to display intermediary phenotypes of biofilm stability and sliding on agar surface [38]. *M. smegmatis* growth as biofilm was associated with upregulation of GPL biosynthetic genes and other gene products required for supplying resources to this very pathway. However, no reports have appeared till date showing the status of the GPL content at the cell surface in a biofilm mode of growth. We have compared the total deacylated GPLs of mc^2155 cells form its biofilm cultures to its planktonic forms by TLC and successively by mass spectrometry. Among the apolar GPLs it was observed that species having the methoxylated fatty acid chain with less polarity (m/z = 1188, 1174 spot-3) were present in very low abundance relative to the GPL species having a hydroxylated fatty acid chain (m/z = 1174 spot-4, 1160) (Fig. 1, lanes a and c). Also, the newly discovered hyperglycosylated polar GPLs (m/z = 1334, 1320) [32,36,39] were found to be absent in the lipid extracts from the biofilm cultures, concomitant with the accumulation of under-methoxylated species of mass 1145. Thus, there is an indirect evidence of the down-regulation of *mtf2*, responsible for methoxylating the hydroxyl group in the alkyl chain of the fatty acid [40], together with *gtf3* required for glycosylation of an extra deoxy sugar to the terminal di/tri-methoxylated rhamnose [34,41]. Hence, it is tempting to hypothesize that either more population of under-methylated products accumulates and gets impregnated at the outer surface supporting the above explanation, or that the methoxylated GPLs get preferentially secreted out to the exterior as signals for an

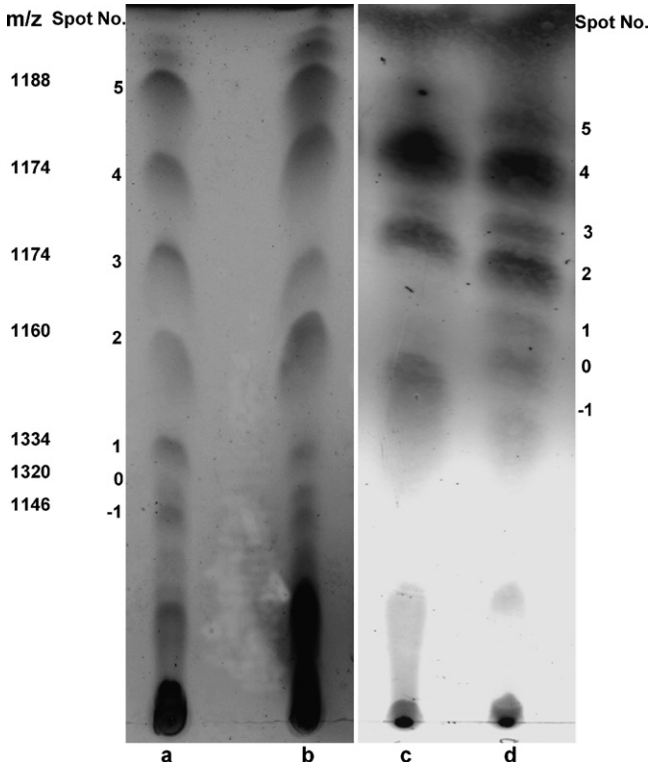

Fig. 1. The total GPL profile of *M. smegmatis* mc^2155 (a, c), mcdrz (b, d) from planktonic (a, b) and biofilm (c, d) cultures shown by TLC. Spot numbers 2, 3, 4, 5 correspond to the apolar GPLs while spots 0 and 1 are of the hyperglycosylated polar GPLs. The m/z values denote the $[M+Na]^+$ of the individual species of GPLs purified by preparative TLC.

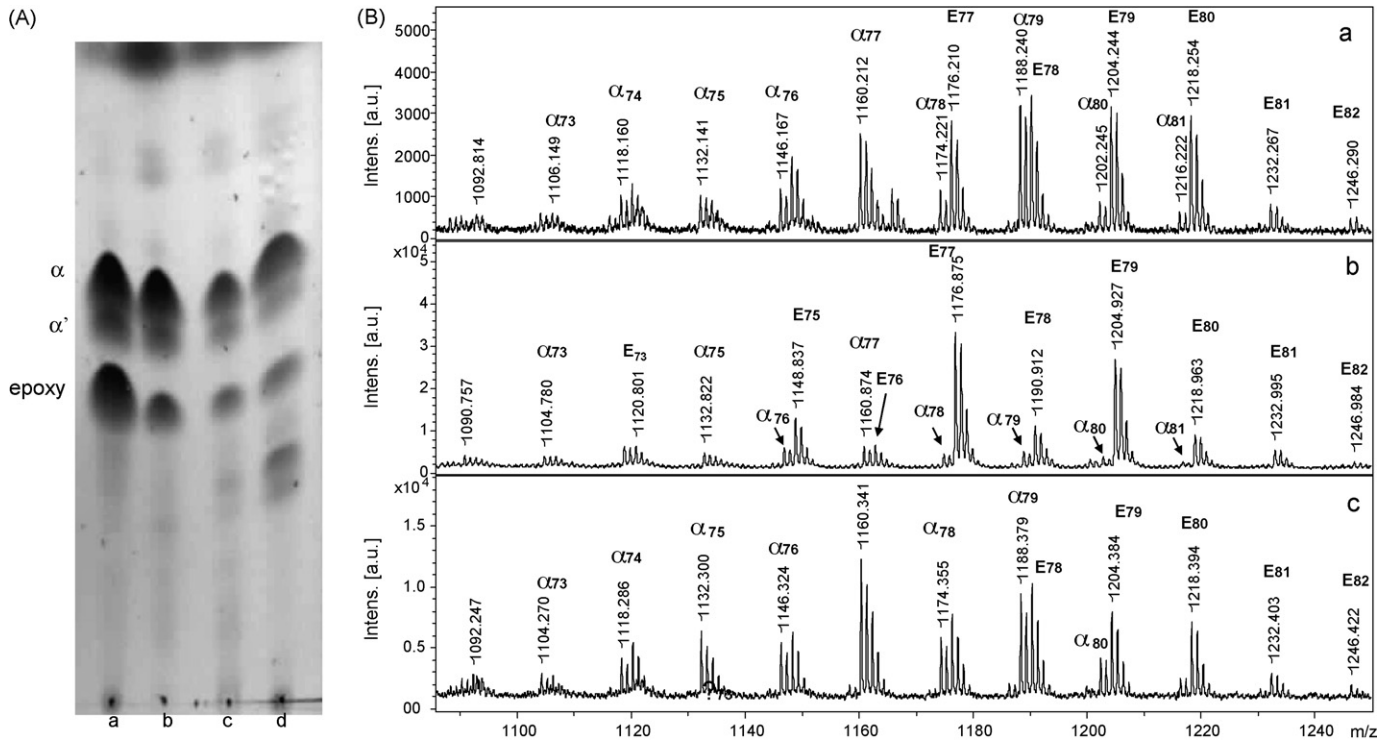

Fig. 2. (A) Thin layer chromatography profile of the total mycolic acid methyl esters, extracted from biofilm cultures of *M. smegmatis* mc^{2}155 (a, b), mcdrz (c, d). Cells harvested at 6 days were from the initiation phase (b, c) and at 8 days (a, d) from a mature biofilm. (B) MALDI-TOF-MS profile of the total α mycolates and the epoxy[E]-mycolates of mc^{2}155 planktonic cells (a), mc^{2}155 cells from biofilm cultures (b) and mcdrz cells from biofilm cultures (c). The number at the subscript denotes the number of carbon atoms in each species of mycolate.

environmental alteration. However, since the local hydrophilicity at the polar head remained unchanged, the increased polarity of the fatty acid chain could perturb the compactness of the GPLs with other neighboring lipids deep inside the cell surface. This may help the cells to remain afloat during the initial process of adherence in a floating biofilm.

3.2. Mycolic acid composition of a mature biofilm

Recently, it has been shown that the level of *pks*10, required for the synthesis of methyl branched fatty acids, increases several folds in *M. avium* biofilms [22]. We have earlier reported that a transition from short-chain mycolates to the actual C_{74}–C_{82} long alkyl chains is associated with biofilm maturation [31]. To detect the precise composition of the thick mycolic acid layer in the cell under biofilm mode of growth, we have used the isotopic distribution of the mycolic acids in an extended resolution MALDI-TOF mass spectrometer. There exists three distinct kinds of mycolic acids in *M. smegmatis*, the longer α and epoxy mycolates and α′ mycolates with a shorter meromycolate chain, while each species has a varying alkyl chain length leading to microheterogeneity [42]. The epoxy mycolates overlap with that of α mycolates due to their mass separation of only 2 Da and can only be distinguished by their isotopic distribution, wherein the tri-isotopic masses of the α mycolates overlap with the epoxy mycolates at its monoisotopic values. We have observed that the hydrophilic epoxy forms of length C_{75}–C_{81} predominate in the mycolic acid methyl ester extracts from a mature biofilm compared to the C_{74}–C_{81} α mycolates (Fig. 2A, lane a), thus increasing the polarity of the mycolate layers. This difference became more prominent in an extended resolution MS spectrum, where the low abundance of the α mycolates was clearly depicted from the spectrum of mc^{2}155 total mycolates of mature biofilms (Fig. 2B, b). However, there was no change in the composition of the shorter α′ mycolates (C_{60}–C_{68}). This is in conjunction to the recruitment of the GPLs, with more hydrophilic alkyl chain, at the cell surface. Since the epoxy group also resides at the long alkyl chain of the mycolic acids, it carries a property similar to the hydroxy GPLs, further to the inner layers of the cell surface. This also reveals plausible distinct functions of the individual class of mycolates.

3.3. rpoZ-deleted mc^{2}155 maintain planktonic growth in floating biofilms

We have reported earlier that *rpoZ*-deleted mc^{2}155 strain has similar total lipids, mycolic acid profile and hydrophobicity as compared with wild-type cells, when grown planktonically [31]. The GPL profile of mcdrz from biofilm cultures was found to contain all the four characterized apolar GPLs, thus being largely similar to that of planktonically grown wild-type and mcdrz cells, together with the presence of the hyperglycosylated polar species (Fig. 1). On comparing the mycolates produced in mcdrz to that of mc^{2}155, it was found to be similar by thin layer chromatography, but the difference was evident by MS analysis which showed the distribution of the α and epoxy mycolates

Table 1
Summary of the proteins characterized in this investigation

Spot no.	Protein/gene ID	Function	Sequence
1	Gnd MSMEG3638	6-Phosphogluconate dehydrogenase, decarboxylating	**SALDLGVPVTGIAEAVFAR** **SETIAEFIDALEKPR** **DYFGAHTYGR** **LPAALIQGLR**
2, 3	MmsA MSMEG2445.1	Methylmalonate-semialdehyde dehydrogenase	**STVIQHWR** **AVIDSAAAAFPAWR** **HHRRYRITEGAEEIQMR** **TQVLFAFR**
4, 5	Ald MSMEG2658	Alanine dehydrogenase	**LSAQVGAYHLMR** **VHDTIFYCVANMPGAVPR** **VAAGMGAHVTVFDLNINTLR** **VAITPAGVAELTR**

Sequences obtained from MALDI-MS-MS spectra are in bold letters.

equally like the planktonically grown mc^{2}155 cultures. It must also be mentioned here that a spot of low mobility was also noted in the TLC profile of mcdrz (Fig. 2A, lane d) but its identity could not be confirmed. mcdrz cells did not show the presence of short-chain mycolates which is believed to constitute the greasy extracellular matrix at the initiation stages of a mycobacterial biofilm development. As the biofilm matures beyond fifth day, both the long-chain mycolates accumulate at the outer layers, unlike their parent strain where mostly the epoxy species inhabit the mycolate layer. Thus it was apparent that the omega-deficient mc^{2}155 cells failed to preferentially recruit the methoxylated apolar GPLs and the hydrophobic mycolates or any yet unknown metabolite to the outer surface, hence were impeded to initiate the biofilm mode of growth from its planktonic existence upon inoculation.

3.4. Proteomic analysis of the rpoZ-deleted mc^{2}155

Since it was shown that an optimal level of properly folded KasA is necessary for production of short-chain mycolates and biofilm maturation [16] we wanted to test the proteome of mcdrz from its biofilm mode of growth by displaying in a 2D PAGE. Upon matching the total cellular proteins of mcdrz with that of wild-type cells, we observed that many of the proteins showed similar profile on a 2D gel (Fig. 3). However, four of the spots were found to be more abundant in mcdrz, their identities were confirmed using MS-MS analysis and were found to be members of the dehydrogenase family (Table 1). Spots 2 and 3 were matched with methyl-malonyl semialdehyde dehydrogenase and spots 4 and 5 were confirmed to be of alanine dehydrogenase, while spot 1, corresponding to 6-phosphogluconate dehydrogenase, was noted to be less abundant than in wild type. Next, we subjected the strain mcdrzco for proteome analysis in the similar way (not shown , presented as a supplementary material). mcdrzco was generated by complimenting *rpoZ* in mcdrz [30], where *rpoZ* was integrated back to the genome presumably at a different location from the original. The 2D profile showed similar pattern as that of wild type (with respect to spots 1, 2 and 3). However, few spots like 4 and 5 did not restore back in the complimented strain, perhaps due to the stress

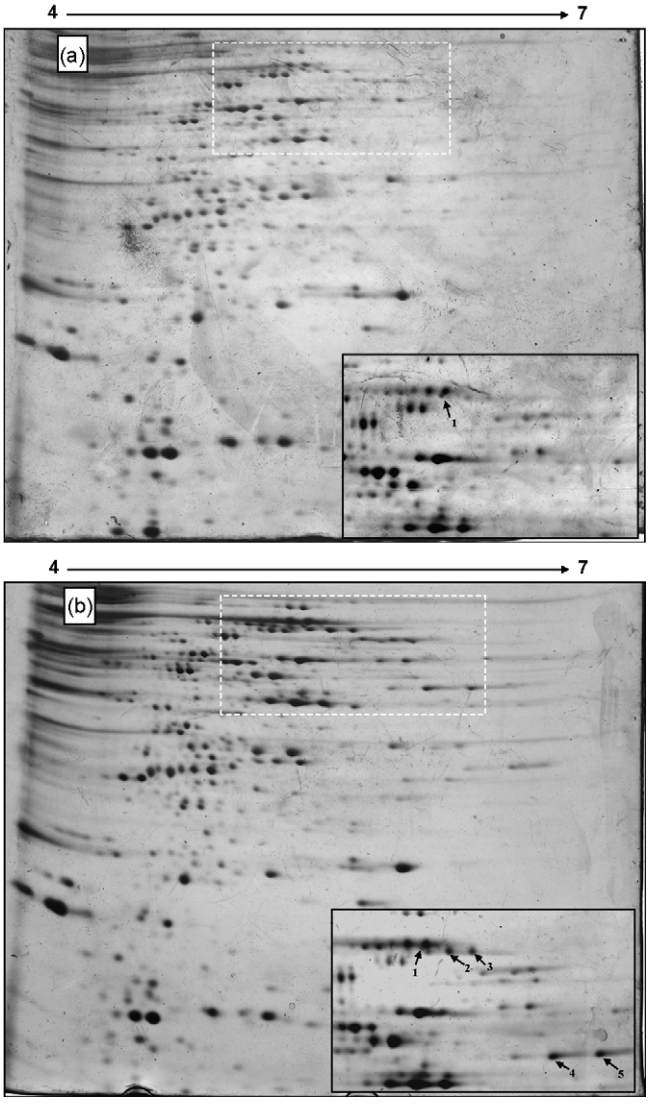

Fig. 3. Total cellular proteomic profile from mc^{2}155 (a) and mcdrz (b) biofilm cultures displayed in a 15% SDS–PAGE. Inset shows the expanded view of the boxed region as observed in a 7.5% SDS–PAGE. The numbers of the protein spots corresponds to that of Table 1.

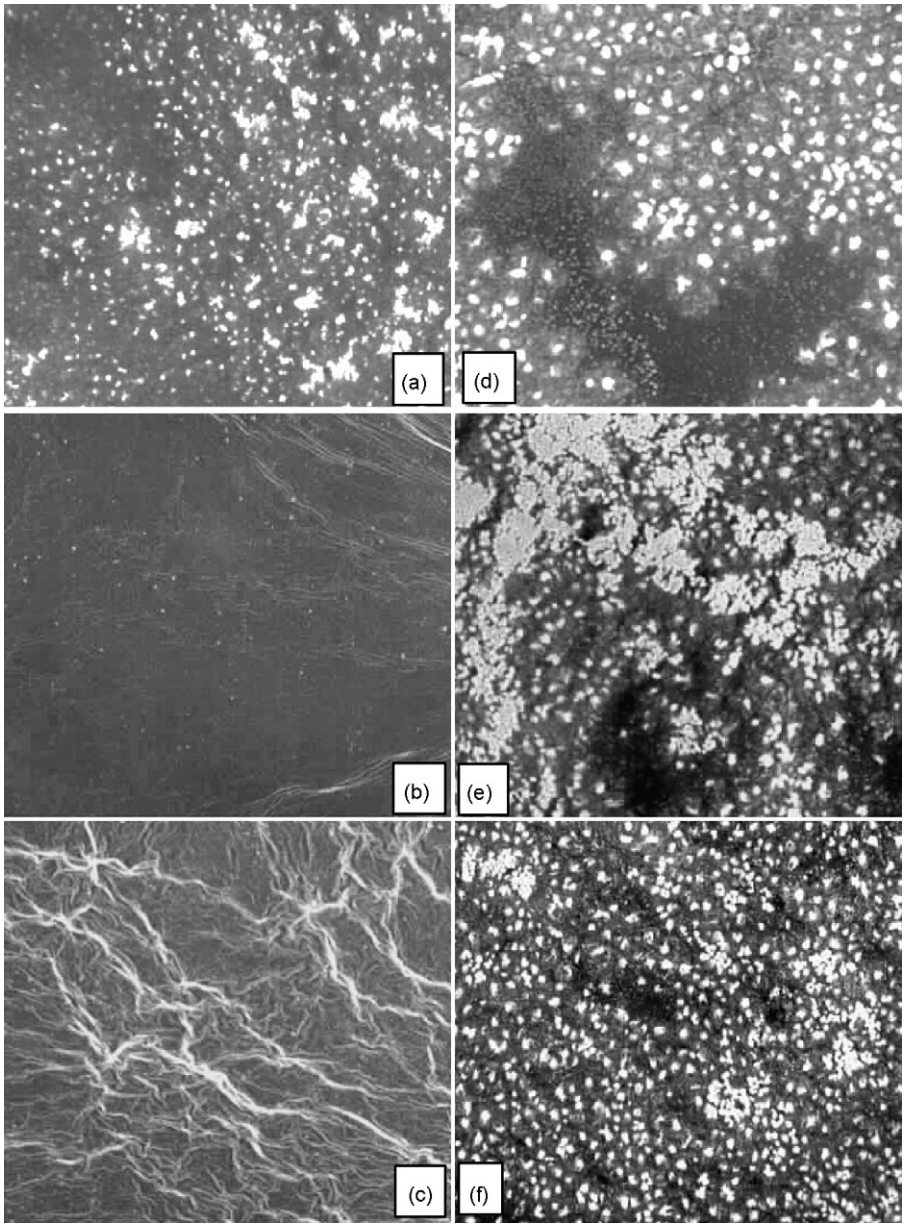

Fig. 4. Developmental stages of mc^{2}155 (a–c) and mcdrz (d–f) biofilms in a polystyrene Petri plate after 4 (a, d), 6 (b, e) and 8 (c, f) days of incubation in humidified incubator. A thin biofilm starts to form by sixth day (b), which matures to form a characteristic wrinkled surface (c).

the cells need to undergo during knockout and then knock-in experiments.

The alternate process of conversion of succinate to acetyl-CoA via the malonate requires the enzyme methyl-malonate semialdehyde dehydrogenase (spots 2 and 3), thus generating acetyl-CoA in cells when conventional pyruvate dehydrogenase activities are low due to limiting oxygen [43]. The *ald* gene was found to be induced fourfold during anaerobic growth in *M. smegmatis* (spots 4 and 5) [44,45]. β oxidation of fatty acids consume lot of NAD^+ during sustained anaerobic growth; therefore to replenish the cellular NAD^+, dehydrogenases act upon pyruvates and NADH to produce NAD^+ [46,47]. However, the downregulation of 6-phosphogluconate dehydrogenase was not unusual as it is a constituent enzyme in one of the processes of consuming D-glucose and subsequently channeling to the hexose mono-phosphate pathway, through the 6-phospho-D-gluconate as an intermediate [48]. Hence it reveals that a near microaerobic growth exists in the mcdrz cells. This is also evident from Fig. 4, which shows the development of mcdrz biofilm in a polystyrene Petri dish. The cells at first grow in clusters of floating colonies, and then they spread like wild-type cells. However, majority of them settle to the lower layers as the growth progresses to the eighth day with concomitant switchover to the relatively microaerobic metabolism.

4. Conclusion

Several studies in recent past [15,16] have shown that GPLs and mycolates play a dominant role in biofilm initiation and maturation in *M. smegmatis*. However, this and reports from other

groups [19] show the complexity of biofilm mode of growth wherein several transcription regulators may have integral role to play. Thus it can be hypothesized that the formation of biofilm takes place in a multistep process with close synchrony among them, where the presence of optimal concentrations of secondary metabolites and some of their novel derivatives is only a prerequisite. The latter process may involve selective transportation or secretion to the exterior to build a quorum, while a compromise in any of the minute processes leads to only retrograde planktonic growth under nutrient limitation. In our effort to uncover the less studied mycobacteria–environment interactions, mcdrz is exemplary of having an early defect in the phenomenon.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2007.08.009.

References

[1] S.S. Branda, S. Vik, L. Friedman, R. Kolter, Trends Microbiol. 13 (2005) 20.
[2] L. Hall-Stoodley, P. Stoodley, Curr. Opin. Biotechnol. 13 (2002) 228.
[3] K. Lewis, Nature 5 (2007) 48.
[4] L. Hall-Stoodley, J.W. Costerton, P. Stoodley, Nat. Rev. Microbiol. 2 (2004) 95.
[5] L. Hall-Stoodley, P. Stoodley, Trends Microbiol. 13 (2005) 7.
[6] J.W. Costerton, P.S. Stewart, E.P. Greenberg, Science 284 (1999) 1318.
[7] L. Marsollier, T. Severin, J. Aubry, R.W. Merritt, J.P. Saint Andre, P. Legras, A.L. Manceau, A. Chauty, B. Carbonnelle, S.T. Cole, Appl. Environ. Microbiol. 70 (2004) 6296.
[8] L. Marsollier, T. Stinear, J. Aubry, J.P. Saint Andre, R. Robert, P. Legras, A.L. Manceau, C. Audrain, S. Bourdon, H. Kouakou, B. Carbonnelle, Appl. Environ. Microbiol. 70 (2004) 1097.
[9] A.B. Kressel, F. Kidd, Infect. Control Hosp. Epidemiol. 22 (2001) 414.
[10] G. Carter, M. Wu, D.C. Drummond, L.E. Bermudez, J. Med. Microbiol. 52 (2003) 747.
[11] M. Dailloux, M. Albert, C. Laurain, S. Andolfatto, A. Lozniewski, P. Hartemann, L. Mathieu, Appl. Environ. Microbiol. 69 (2003) 6946.
[12] E. Bardouniotis, H. Ceri, M.E. Olson, Curr. Microbiol. 46 (2003) 28.
[13] L. Hall-Stoodley, H. Lappin-Scott, FEMS Microbiol. Lett. 168 (1998) 77.
[14] S. Lustgarten, Ueber spezifische Bacillen in syphilitische Krankheitsproduckten, Weiner Medizinische Wochenschrift 1 (1984).
[15] J. Recht, A. Martinez, S. Torello, R. Kolter, J. Bacteriol. 182 (2000) 4348.
[16] A. Ojha, M. Anand, A. Bhatt, L. Kremer, W.R. Jacobs Jr., G.F. Hatfull, Cell 123 (2005) 861.
[17] C. Beloin, J.M. Ghigo, Trends. Microbiol. 13 (2005) 16.
[18] L. Rose, S.H. Kaufmann, S. Daugelat, Microbes Infect. 6 (2004) 965.
[19] J.M. Chen, G.J. German, D.C. Alexander, H. Ren, T. Tan, J. Liu, J. Bacteriol. 188 (2006) 633.
[20] G. O'Toole, H.B. Kaplan, R. Kolter, Annu. Rev. Microbiol. 54 (2000) 49.
[21] B.M. Pru, C. Besemann, A. Denton, A.J. Wolfe, J. Bacteriol. 188 (2006) 3731.
[22] Y. Yamazaki, L. Danelishvili, M. Wu, M. MacNab, L.E. Bermudez, Appl. Environ. Microbiol. 72 (2006) 819.
[23] S. Rachid, K. Ohlsen, U. Wallner, J. Hacker, M. Hecker, W. Ziebuhr, J. Bacteriol. 182 (2000) 6824.
[24] K.D. Xu, M.J. Franklin, C.H. Park, G.A. McFeters, P.S. Stewart, FEMS Microbiol. Lett. 199 (2001) 67.
[25] M. Kalmokoff, P. Lanthier, T.L. Tremblay, M. Foss, P.C. Lau, G. Sanders, J. Austin, J. Kelly, C.M. Szymanski, J. Bacteriol. 188 (2006) 4312.
[26] K. Sauer, A.K. Camper, G.D. Ehrlich, J.W. Costerton, D.G. Davies, J. Bacteriol. 184 (2002) 1140.
[27] A. Martinez, S. Torello, R. Kolter, J. Bacteriol. 181 (1999) 7331.
[28] R.R. Burgess, J. Biol. Chem. 244 (1969) 6168.
[29] P. Ghosh, A. Ishihama, D. Chatterji, Eur. J. Biochem. 268 (2001) 4621.
[30] R. Mathew, M. Ramakanth, D. Chatterji, J. Bacteriol. 187 (2005) 6565.
[31] R. Mathew, R. Mukherjee, R. Balachandar, D. Chatterji, Microbiology 152 (2006) 1741.
[32] R. Mukherjee, M. Gomez, N. Jayaraman, I. Smith, D. Chatterji, Microbiology 151 (2005) 2385.
[33] L. Kremer, J.D. Douglas, A.R. Baulard, et al., J. Biol. Chem. 275 (2000) 16857.
[34] R. Mukherjee, D. Chatterji, Biochem. Biophys. Res. Commun. 338 (2005) 964.
[35] M. Daffe, M.A. Laneelle, G. Puzo, Biochim. Biophys. Acta 751 (1983) 439.
[36] P.J. Brennan, M.B. Goren, J. Biol. Chem. 254 (1979) 4205.
[37] R. Freeman, H. Geier, K.M. Weigel, J. Do, T.E. Ford, G.A. Cangelosi, Appl. Environ. Microbiol. 72 (2006) 7554.
[38] J. Recht, R. Kolter, J. Bacteriol. 183 (2001) 5718.
[39] A.K. Ojha, S. Varma, D. Chatterji, Microbiology 148 (2002) 3039.
[40] D. Jeevarajah, J.H. Patterson, M.J. McConville, H. Billman-Jacobe, Microbiology 148 (2002) 3079.
[41] C. Deshayes, F. Laval, H. Montrozier, M. Daffe, G. Etienne, J.-M. Reyrat, J. Bacteriol. 187 (2005) 7283.
[42] M.Y.H. Wong, P.A. Steck, G.G.R. Gray, J. Biol. Chem. 254 (1979) 5734.
[43] D.C. Pauling, J.P. Lapointe, C.M. Paris, R.A. Ludwig, Microbiology 147 (2001) 2233.
[44] Z. Feng, N.E. Cáceres, G. Sarath, R.G. Barletta, J. Bacteriol. 184 (2002) 5001.
[45] M.I. Voskuila, K.C. Viscontib, G.K. Schoolnik, Tuberculosis 84 (2004) 218.
[46] D. Schnappinger, S. Ehrt, M.I. Voskuil, Y. Liu, J.A. Mangan, I.M. Monahan, G. Dolganov, B. Efron, P.D. Butcher, C. Nathan, G.K. Schoolnik, J. Exp. Med. 198 (2003) 693.
[47] I. Rosenkrands, R.A. Slayden, J. Crawford, C. Aagaard, C.E. Barry III, P. Andersen, J. Bacteriol. 184 (2002) 3485.
[48] C. Stournaras, P. Maurer, G. Kurz, Eur. J. Biochem. 130 (1983) 391.