electrophoresis และ Southern blot ลงบน nylon membrane hybridize ด้วย probe จาก *arr* gene PCR product ของ *M. smegmatis* และ PCR product ของตัวเอง พบว่าตำแหน่งที่ให้ band เป็นตำแหน่งเดียวกันคือของ *M. fortuitum* และ *M. chelonae* อยู่ที่ 2.5 kb, *M. smegmatis* อยู่ที่ 6 kb (รูปที่ 4)ทำให้น่าสงสัยว่ากลุ่ม fast growing bacteria นี้น่าจะมี *arr* gene ที่สนใจอยู่ ซึ่ง MICของ *M. chelonae* มีค่า >90 μg/ml และ MIC ของ *M. fortuitum* มีค่า 40 μg/ml

ทำการศึกษาลำดับเบสของ gene ที่สงสัยโดยนำ PCR product ที่ได้มา sequence. พบว่า ลำดับเบสที่ได้จาก *M. aurum* MNC979 และ *M. smegmatis 14468* เหมือนกันกับ *arr* gene ของ *M. smegmatis* DSM 43756 เกือบ 100 % (รูปที่ 5)

ส่วนลำดับเบสที่ได้จาก *M. chelonae* และ *M. fortuitum* นั้นมีความเหมือนกันกับ arr gene ของ *M. smegnatis* DSM 43756 48.59 % และ 51.57 % ตามลำดับ ดังแสดงในรูปที่ 5

แต่เนื่องจาก PCR product ที่ได้ ไม่ครอบคลุม start และ stop codon ของสาย gene จึงทำการ ตัด band ของ DNA ที่ให้ผลบวกต่อ *arr* gene ไปต่อเข้าใน plasmid pMV 261 transform เข้าใน *E.coli* DH5α เลือก clone ใน kanamycin plate นำไป sequence หาลำดับ gene ที่สมบูรณ์ต่อไปและนำ clone ที่ได้ไปศึกษา phenotype ใน *M. smegmatis* mc^2 155 เพื่อดูความสามารถในการดื้อยา (อยู่ในระหว่างทำการศึกษา)

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Arr      1   GTGGTGGCGAATCCGCCGAAACCGTTCGAAGTGCACGAGTCCGGGGGCCTATCTGCACGGCACCAAG 66
aureum   1                                TGC-CGAGCCCGGGGGCCTATCTGCACGGCACCAAG 34

Arr      67  ACCGACCTCAAGGTGGGGGACCGACTGGTGCCCGGCCGCGAGTCCAACTTCGAGGCCGGGCGCATC 132
aureum   35  ACCGACCTCAAGGTGGGGGACCGACTGGTGCCCGGCCGCGAGTCCAACTTCGAGGCCGGGCGCATC 100

Arr      133 ATGAAGCACGTCTACATCACCCAGACGCTGGACGCCGCGGTGTGGGGCGCCGAGCTTGCTGTCGGT 198
aureum   101 ATGAAGCACGTCTACATCACCCAGACGCTGGACGCCGCGGTGTGGGGCGCCGAGCTTGCTGTCGGT 166

Arr      199 GAGGGTCGCGGGCGGATTTACATCGTCGAACCCGAGGGCGAGATCGAAGACGACCCGAACGTCACC 264
aureum   167 GAGGGTCGCGGGCGGATTTACATCGTCGAACCCGAGGGCGAGATCGAAGACGACCCGAACGTCACC 232

Arr      265 GACAAGAAGCTCCCCGGCAACCCGACCCGCTCCTACCGCACCCGTGAGCCCGTGCGGATCGTCGGG 330
aureum   233 GACAAGAAGCTCCCCGGCAACCCGACCCGCTCCTACCGCACCCGTGAGCCCGTGCGGATCGTCGGG 298

Arr      331 GAGCTCACCGACTGGGAGGGGCATTCGCCGGAGCAGATCGCTGCCATGCGGGAGGGGCTCGAGGAT 396
aureum   299 GAGCTCACCGACTGGGAGGGGCATTCGCCGGAGCAGATCGCTGCCATGCGGGAGGGGCTCGAGGAT 364

Arr      397 CTACGGCGCAAGGGGCTCGCGGTCATCTATGACTAG 432
aureum   365 CTACGGCGCAAGGGGCTCGA                 384

Arr        1     GTGGTGGCGAATCCGCCGAAACCGTTCGAAGTGCACGAGTCCGGGGCCTATCTGCACGGCA 61
Chelonae   1   GTCACGCTCG-GCGTCCGTCTTGGTCATACCGGGTCCACG-GTCGATGACCTCGAGG-ACGGCG 61

Arr       62   CCAAGACCGACCTCAAGGTGGGGGACCGACTGGTGCCCGGCCGCGAGTCCAACTTCGAGGCCGG 125
Chelonae  62   TCATCGCCCTCGGTACCGACCCGGACCCGCACTGCGGCGGTGGTGTGACCCAATGCGTTGCT-- 123

Arr       126  GCGCATCATGAAGCACGTCTACATCACCCAGACGCTGGACGCCGCGGTGTGGGGCGCCGAGCTT 189
Chelonae  124  ---CACCA-GATTGCCGAGGACCTGACGTAGCC--TGAGCTCATCGCCGATCACCTCCG-GAGT 180

Arr       190  GCTGTCGGTGAGGGTCGCGGGCGGATTTACATCGTCGAACCCGAGGGCGAGATCGAAGACGACC 253
Chelonae  181  GCCCGGGCCATCGAACACCTCCAGTTCCACATCA-CGTTC--GGGGGCAGTGGCTTTCGCGTCG 241

Arr       254  CGAACGTCACCGACAAGAAGCTCCCCGGCAACCCGACCCGCTCCTACCGCACCCGTGAGCCCGT 317
Chelonae  242  TGAACGGCGTCGGTGGCCAGCACC--AGCAAATCGACCAACCGCTGCTCCA----TGGGGCGAT 299

Arr       318  GCGGATCGTCGGGGAGCTCACCGACTGGGAGGGGCATTCGCCGGAGCAGATCGCTGCCATGCGG 381
Chelonae  300  GCG---CGTCCAGCCGCG--CCAAC----AGCAGCAGATCCTCCACCAGTTGTC--CCATGCGG 352

Arr       382  GAGGGGCTCGAGGATCTACGGCGCAAGGGGCTCGCGGTCATCTATGACTAG 432
Chelonae  353  AGGGGGCTCGA                                         363


Arr         1    GTGGTGGCGAATCCGCCGAAACCGTTCGAAGTGCACGAGTCCGGGGCCTATCTGCACGGCACC 63
Fortuitum   1                 CCTCGGGCACTTCGTCCCCCGCAAGACTCAGGTGGCCAT---CAATGT-CT 47

Arr         64   AAGACCGACCTCAAGGTGGGGGACCGACTGGTGCCCGGCCGCGAGTCCAACTTCGAGGCCGGG 126
Fortuitum   48   ATG-----CCTCCATGCGGCTTTCCGACTGGTGGCCCGATCCGGA--CACGTTCAATCCCGAA 103

Arr         127  CGCATCATGAAGCACGTCTACATCACCCAGACGCTGGACGCCGCGGTGTGGGGCGCCGAGCTT 189
Fortuitum   104  CGGTTCGTGAA---------CAACGCCGCAACCCG---CGCGGCGGTGAGCCGCTATGTGTTC 154

Arr         190  GCTGTCGGTGAGGGTCGCGGGCGGATTTACATCGTCGAACCCGAGGGCGAGATCGAAGACGAC 252
Fortuitum   155  GC-GCCCTTCGGCGGCGCGCACAAATG--CATCGGCCAGCAGTTCGCCGACAT-GAC--CGTC 211

Arr         253  CCGAACGTCACCGACAAGAAGCTCCCCGGCAACCCGACCCGCTCCTACCGCACCCGTGAGCCC 315
Fortuitum   212  AAGACCACCATGCACCAGATGCTGC--GGCGTTTCGAGTGGAGCGTGCCGAAGAACT-ACCGC 271

Arr         316  GTGCGGATCGTC-GGGGAGCTCACCGACTGGGAGGGGCATTCGCCGGAGCAGATCGCTGCCAT 377
Fortuitum   272  CTGCAGCTGACCTGGGGAACCGGGCCAATGCCCGCCG-AT--GACCTACC-GATCGA---CAT 327

Arr         378  GCGGGAGGGGCT-CGAGGATCTACGGCGCAAGGGGCTCGCGGTCATCTATGACTAG 432
Fortuitum   328  GCGGAGGGGGCTTCGA                                         343
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รูปที่ 5. เปรียบเทียบลำดับเบสของ *arr* gene ที่เพิ่มขยายโดยวิธี PCR ใน *Mycobacterum aurum, M. chelonae, M. fortuitum* กับ *M. smegmatis* DSM 43756 (*arr*) ลำดับเบสที่เหมือนกันแสดงด้วยสีเทา

บทวิจารณ์

จากการที่มีผู้ศึกษาพบ ribosylation เป็นหนึ่งในวิธีการ inactivate ยา rifampicin ใน *Mycobacteria smegmatis* (15) และยังพบใน fast growing mycobacteria อื่นเช่น *M. chelonae* subsp.abscessus IFM 0359, *M. parafortuitum* ATCC 19696 ซึ่งเชื้อเหล่านี้มี MIC ต่อยาสูงถึง 50 μg/ml จากนั้นการศึกษาพบ gene ที่แสดงออกถึง rybosylating activity ที่มีขนาด 429 bpใน *M. smegmatis* (20) ที่ชื่อ arr gene (ADP-ribosyl transferase gene ต่อมา น.พ.ชาญวิทย์ ตรีพุทธรัตน์ รายงาน rifampicin resistant gene ที่ให้ชื่อว่า *arr*-2 จาก *Pseudomonas aeruginosa* สายพันธุ์จากประเทศไทย ซึ่งมีความเหมือนกับ *arr* gene ถึง 43% ซึ่ง locate อยู่บน class I integron (18) จึงน่าสนใจว่า gene นี้อาจส่งผ่านให้ Mycobacteria ที่ดื้อยา rifampicin หรือไม่

จึงทำการตรวจหา gene ที่ homology กับ *arr*-2 geneใน *Mycobacteria* โดยวิธี Southern bolt hybridization แต่ไม่พบ band ซึ่งเป็นไปได้ว่า ribosyl transferase gene ใน Mycobacteria เหล่านี้มีความต่างกับ *arr*-2 gene ของ *Pseudomonas aeruginosa*

อย่างไรก็ตาม โดยวิธี PCR และ dot blot hybridization สามารถตรวจหา gene ที่ complementary กับ *arr* gene ของ *M. smegmatis* DSM43756 ได้ใน *M. smegmatis* ATCC1468 แต่ไม่พบใน *M. smegmatis* 16941 และพบใน *M. aurum* MNC979 บางสายพันธุ์ของ *M. fortuitum* และ *M. chelonae* ที่แยกได้จากผู้ป่วย ซึ่งเป็นกลุ่ม fast growing mycobacteria ในสิ่งแวดล้อมเช่นเดียวกับ *M. smegmatis* โดยเฉพาะ *M. aurum* MNC979 นั้นมี arr gene ที่มีลำดับ sequence ตรงกับ ของ *M. smegmatis* geneนี้อาจเป็น gene ที่สามารถส่งผ่านกันระหว่าง bacteria ที่มีผลทำให้มีการดื้อยาในธรรมชาติเกิดขึ้น

การดื้อยาปฏิชีวนะของ *M. smegmatis* และ *M. fortuitum* มักมีความสัมพันธ์กัน เช่น การพบ aminoglycoside-2'-*N*-acetyltransferase genes ใน *M. smegmatis* และใน *M. fortuitum* ซึ่งมีลำดับโปรตีนเหมือนกันเพียง 38 % (23) ดังนั้น ความเหมือนกันของ ribosylation gene อาจมีไม่มากนักซึ่งต้องทำการศึกษา phenotype ให้สมบูรณ์ต่อไปเพื่อยืนยันคุณสมบัตินี้

บทสรุป

พบ arr gene ใน *M. aurum* ซึ่งมีลำดับ amino acid ตรงกันกับ *arr* gene ที่พบใน *M. smegmatis* DSM.43756 พบลำดับ sequence ที่คล้าย *arr* gene ใน *M. fortuitum* และ *M. chelonae* ทำให้น่าสงสัยว่าอาจมีผลต่อการดื้อยา riformpicin ของสายพันธุ์ดังกล่าว และไม่พบใน *M. tuberculosis* และใน *Mycobacerium avium complex* ซึ่งการลดฤทธิของยาด้วยวิธี ribosylation อาจไม่มีบทบาทในสายพันธ์เหล่านี้

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Out put

Suporn Foongladda,Chulabha Tullatum, Wiyada Arjrattanakul, and Angkana Chaiprasert. Detection of rifampicin ADP-ribosyl transferase (*arr*) gene in *Mycobacteria* species. 2000; (submitted to Antimicrob Agents and Chemother)

Detection of rifampicin ADP-ribosyl transferase (*arr*) gene in *Mycobacteria* species

Suporn Foongladda,* Chulabha Tulllatum, Wiyada Arjrattanakul, and

Angkana Chaiprasert

Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University,

Bangkok, Thailand

* Corresponding author. Mailing address:

Department of Microbiology, Faculty of Medicine,

Siriraj Hospital, Mahidol University,

2 Prannok, Bangkoknoi,

Bangkok 10700, Thailand.

Phone: (662) 419-7062

Fax: (662) 411-3106

E-mail: sisfm@mahidol.ac.th

Abstract

DNA extract of mycobacteria 19 reference strains and clinical isolates of 127 *M. avium* complex, 35 *M. chelonae*, 12 *M. fortuitum*, 20 rifampicin resistant *M. tubterculosis* and 94 rifampicin sensitive *M. tubterculosis* were used to detect Rifampin ADP-ribosyl transferase (*arr*) gene by PCR and dot blot hybridization. The *arr* of *M. smegmatis* DSM43756 homology genes were detected in *M. smegmatis* ATCC14468, *M. aurum* MNC979, 34/35 clinical isolates of *M. chelonae* and 8/12 clinical isolates of *M. fortuitum*. The DNA sequence of *arr* gene from *M. aurum* MNC979 and *M. smegmatis* ATCC14468 were identity to that was reported in *M. smegmatis* DSM43756, but that from *M. chelonae* and *M. fortuitum* show only 48.59 % and 51.57 % identity, respectively.

Introduction

Rifampicin is a major drug for the treatment of mycobacterial infection (1). Rifanpicin resistant mechanism of *Mycobacterial tuberculosis* has been shown predominantly associated with the mutation at one of three loci in *rpo B* gene. However, rifampicin resistant *M. tuberculosis* with no *rpo B* mutation have been reported (2,3,4,5). In addition, *M. aviun* complex and *M. smegmatis* did not have any mutations in *rpo B* gene (6,7). Ribosylation activity have been reported in fast growing mycobacteria, including, *M. smegmatis*, with high MICs of rifampicin (8). ADP-ribosyl transferase (*arr*) gene (449 bp) responsible for this ability was shown in *Mycobacterium smegmatis* DSM43756 (Gen-bank accession #AF 001493)(9). Recently, arr-2 gene has been found in clinical Thai isolated *Pseudomonas aeruginosa* that is located on an integron (10). This gene might have been transferred between bacteria in the environment in soil and might have been transfer between mycobacteria. The aim of this study was to detect *arr*-like gene in *Mycobacteria* referent strains and in clinical strains.

Matherials and methods

Bacterial strains, plasmid and growth conditions: The reference strains used in this study are *M. smegmatis* DSM43756 (positive control of *arr* gene), *M. smegmatis* 16941, *M. smegmatis* ATCC14468, *M. vaccae* MNC450, *M. bovis* LCDC 302, *M. bovis* BCG700, *M. gordonae* ATCC14470, *M. microti* LCDC201, LCDC203, KK1401, *M. kansasii* ATCC 12478, *M. flavescen* ATCC23035, *M. ulcerans* KK43-02, *M. africanum* 501, *M. aurum* MNC979, *M. austoafricanum*, *M. duvalii* MNC442, *M. fortuitum 7.2*, *M. chelonae* 0019, *M. tuberculosis* 12011-41 and T2-TBD-0643, *M. tuberculosis* H37Rv (India strain and Japan

strain) and *M. tuberculosis* K37Rv. One hundred and twenty seven clinical isolates of *M. avium* complex, 35 clinical isolates of *M. chelonae*, 12 clinical isolates of *M. fortuitum*, 20 clinical isolates of rifampicin resistant *M. tubterculosis* and 94 clinical isolates of rifampicin sensitive *M. tubterculosis* were obtained from the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University. *M smegmatis* mc^2 155 and *E. coli* DH5α were used in the transformation procedure. Loewenstein-Jensen medium (Becton Dickinson, USA) was used to culture the mycobacterial strains. *E. coli* DH5α was culture in L-B medium. All cultures were incubated at 37 °C. Kanamycin (50 μg/ml) or rifampicin (20 μg/ml) (Sigma Chemical Co.,USA) were added when necessary. Plasmid, pPMV261, was maintained in *E. coli* and selected by using 50 μg of kanamycin per ml.

DNA isolation. Genomic DNA of mycobacteria was isolated by mechanical lysis and enzymatic lysis as described by van Soolingen *et al* (11). Three-four weeks old cultures of mycobacterial cells were lysed by vortexing in 0.5 ml TE-10-1 (1o mM Tris, 1mM EDTA) buffer with 8-10 glass beads (ϕ 5 mm) and then by adding lysozyme (Gibgo BRL) to a final concentration of 10 mg/ml and incubated at 37 °C for over night. Cells were lysed after the addition of Proteinase K (Sigma) and 10% SDS to a final concentration of 0.1 mg/ml and 1%, respectively. The mixtures were incubated at 65 °C for 10 min followed by the addition of 0.15 ml 5 M NaCl and 0.15 ml CTAB (N-acetyl-N,N,N-trimeylammonium bromide, Sigma) solution (0.1 g/L CTAB, 0.041 g/L NaCl). DNA were following extracted by Phenol-chloroform-isoamyl alcohol and by chloroform-isoamyl alcohol. Following DNA precipitation with 95% ethanol and washing with 70% ethanol, DNA pellets were collected and resuspended in TE10-1 at –20 °C.

DNA manipulation. DNA were purified after electrophoresis through low-melting-temperature agarose (SeaPlaque, FMC, USA). After excision the agarose was melted at 65 °C and DNA purification was performed by using QIAquick Gel Extraction kit from Qiagen, USA. Electrophoresis were perform as described elsewhere and according to the supplier's recommendations (FMC, Gibco BRL, New England, USA)

PCR. The pair of primers results in an expected PCR product (382 nt) that spans the site of the *arr* gene of *M. smegmatis* DSM43756 as published previously (9). The primers P1 (5'-ATC CGC CGA AAC CGT TCG-3') and P2 (5'-TCG AGC CCC TCC CGC ATG-3'), were designed from sequence submitted to GenBank (accession number AF001439).

DNA was amplified in the buffer supplied by the *Taq* polymerase manufacturer (Gibgo BRL, Grand Island, N.Y., USA) in a 50-µl volume containing 0.8 µM primers, 1.5 mM $MgCl_2$, 2.5 mM of each dNTP (Amresco, USA) and 2 U of *Taq* polymerase. The reactions were performed with an automated thermal cycle (Perkin-Elmer Cetus, USA). DNA were denatured by incubation for 3 min at 94 °C before 30 cycle of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 mim. Ten microliters of the PCR products were analyzed by electrophoresis through a 2% agarose gel (SeaKem; FMC Bioproducts, USA) in TAE buffer (40 mM Tris-acetate, 0.2 mM EDTA). Bands were visualized by UV translumination after ethidium bromide staining.

Hybridizations: For dot blot and southern blot hybridization, DNA from the *Mycobacterium* strains were transferred to nylon filters (hybond N: Amersham, England) as described previously (12). The probes were labeled by random primer labeling (Gene Images random prime labelling module, Amersham, England) with fluorescein-11-dUTP.

Prehybridization and hybridization were carried out in Rapid Hybridization Buffer (Amersham) at 50 °C for 60 mim and overnight, respectively. Filters were washed at 55 °C twice with 1x SSC (1XSSC is 0.15 M NaCl plus 0.015M sodium citrate) plus 0.1% SDS, and once with 0.5xSSC plus 0.1% SDS. The blocking stage was carried out in Liquid block agent (Gene Images CDC-Stardetection module, Amersham, England) in 0.1M Tris-HCl -0.3 M NaCl pH9.5 at room temperature for 1 hour. After a blocking step, the filters were incubated with an antifluorescein alkaline phosphatase conjugate in 0.5% bovine serum albumin in 0.1M Tris-HCl -0.3 M NaCl pH9.5 at room temperature for 1 hour. After washing of the excess conjugate at room temperature 3 times with 0.1M Tris-HCl - 0.3 M NaCl pH9.5 - 0.3 Tween20, probe bound AP was detected by decomposition of the dioxetane substrate in Detection reagent (Amersham) for 2-5 min and exposed on the X-ray film (Kodak, USA).

DNA sequencing. DNA and PCR products were used to purify by QIAquick Gel Extraction (Qiagen, USA) as described by the manufacturer before sequencing. Double-strand DNA sequencing was performed by the cycle sequencing with the ABI Prism BigDye Terminator Cycle Squencing Ready Reaction Kit (Perkin-Elmer Applied Biosystem, USA) with P1 primer according to the manufacturer's instruction.

Antibiotic susceptibility testing. The MICs of rifampicin were determined by agar dilution method on Middle brook 7H10 agar (Difco, USA). Ten µl of 10^3 and 10^4 CFU bacteria were spotted on plates containing rifampicin in twofold dilutions. The lowest concentration of rifampicin at which no visible growth was observed after incubation at 37 °C was taken as the MIC level.

Compeuter analysis of sequence data. Nucleotide and amino acid sequences were analyzed and compared by using the DN*Assist* Version 1.01 of Hugh Patterton. Databases were search with the program Blastncbi of the Nation Center for Biological Information.

Results

Detection of the *arr* gene in *Mycobacteria* species. No hybridization was detected between total DNA of many strains of Mycobacteria and probe of *arr*-2 PCR product. PCR were performed with 19 *Mycobacterium* referent strains, 127 clinical isolates of *M. avium* complex, 35 clinical isolates of *M. chelonae*, 12 clinical isolates of *M. fortuitum*, 20 clinical isolates of rifampicin resistant *M. tubterculosis* and 94 clinical isolates of rifampicin sensitive *M. tubterculosis*. PCR products, 382 bp, have been found in *M. smegmatis* DSM 43756, *M. smegmatis* ATCC14468, *M. aurum* MNC979, 34/35 clinical strain of *M. chelonae*, and 8/12 clinical strain of *M. fortuitum* (Fig. 1, 2). The PCR product was not observed in 127 clinical isolates of *M. avium* complex, 20 strains of rifampicin-resistant and 94 of clinical strains *M. tuberculosis*. PCR product (382 nt) of *arr* gene from *M. smegmatis* DMS 43756 was used as a probe in hybridization experiments with these *Mycobacteria* species. Dot blot hybridizations show positive results with those positive PCR strains (Fig. 3). *M. fortuitum*, *M. chelonae* and *M. smegmatis* DSM 43756 were chosen, and their DNAs were digested with BamHI and EcoRI before transfer to a nylon membrane. Southern blot hybridization showed that *M. fortuitum* clinical strains, *M. chelonae* clinical strains and *M. smegmatis* DSM 43756 hybridized in the different bands, 2.5, 2.5 and 6 kb respectively (Fig 4), suggesting that these fast growing

mycobacterial species may have genes homologous to *arr* gene. Hybridized bands showed the same level with those from self PCR probe (data was not shown). MIC of of *M. chelonae* was > 90 μg/ml and MIC of *M. fortuitum* was 40 μg/ml.

DNA sequence analysis. The sequences of the PCR products of *M. fortuitum*, *M. chelonae*, *M. aurum* MNC979 and *M. smegmatis* ATCC14468 were determined. The G+C content of these genes (65%) is in concordance with the values described for mycobacteria genomes (62-70%). *M. aurum* MNC979 and *M. smegmatis* ATCC14468 DNA sequences were identity with *arr* of *M. smegmatis* DSM43756, but that from *M. chelonae* and *M. fortuitum* show only 48.59 % and 51.57 % identity, respectively (Fig 5).

Discussion

Ribosylation is one of an inactivatin of rifampicin by *Mycobacteria smegmatis* (8) and in other fast growing mycobacteria such as, *M. chelonae* subsp.abscessus IFM 0359, *M. parafortuitum* ATCC 19696. MICs of rifampicin of these strains were high as 50 μg/ml. ADP-ribosyl transferase gene have been reported in *M. smegmatis* (*arr*) and in *Pseudomonas aeruginosa* (*arr*-2) (8,9,10). This gene was found on an integron which could transfer between bacteria in the environment. Then we suspected this gene might have been in many strains of *Mycobacteria*. This lead us to used the probe derived from the *arr*-2 gene, with no significant results even when the probes were used under low-stringency conditions. This suggests that genes homologous to *arr-2* gene were not shown in these strains.

By PCR and hybridization with *arr* gene of *M. smegmatis*, we could detect *in M. smegmatis* ATCC1468 and *M. aurum* MNC979 but did not find in *M. smegmatis* 16941.

The finding also include many clinical strains of *M. fortuitum* and *M. chelonae* which are the fast growing group.

Amino sequence of *M. aurum arr* gene was homology with that of *M. smegmatis* DSM.43756. The *arr* like sequences were detected in *M. fortuitum* and *M. chelonae*, which are fast growing mycobacteria in the environment. Anyway, it was not found in *M. tuberculosis* and *Mycobacerium avium* complex. This finding has been shown that *arr* gene could not have in all of the same strain. It was suggested that *arr* gene might transfer between bacteria as a plasmid or integrate into chromosome which need for further research.

The sequences of these genes show highly conserved between *M. aurum* and *M. smegmatis* but show variation in *Pseudomonas aeruginosa* (*arr*-2), *M. fortuitum* and *M. chelonae*. This suggests that sequence of the corresponding gene is not highly conserved that will be further studied to determine whether the resistance is due to the corresponding gene or not. Further studies are being conducted.

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Acknowledgement

We would like to thank Dr Prof. Dr. Yuzuru Mikami, Research Center for Pathogenic Fungi and Microbial Toxicology, Chiba University for supplying *M. smegmatis* DSM43756. We would like to thank the generosity of Dr. Prasit Palittapolkarnpim for his variable advice and supplying pMV261 plasmid.

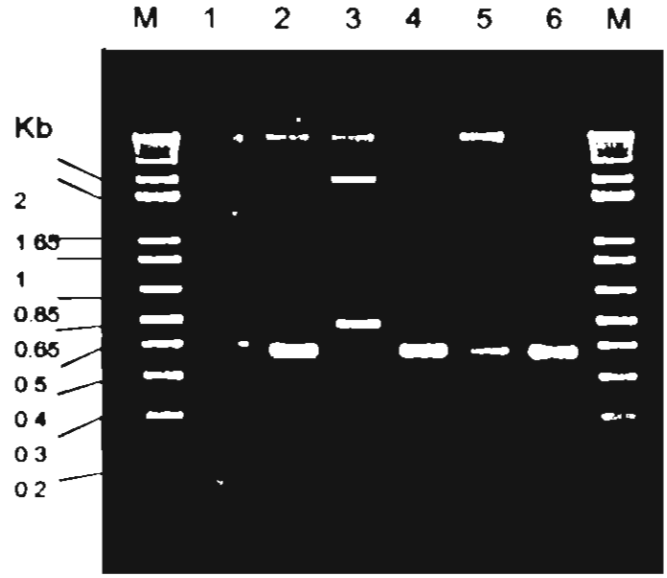

FIG 1. Detection of *arr* gene by PCR of *Mycobacteria*: lane 1 *M. gordonae* ATCC14470, lane 2 *M. smegmatis* ATCC14468, lane 3 *M. duvalei* MNC442, lane 4 and 6 *M. smegmatis* DSM 43756, lane 5 *M. aurum* MNC979, M is a standard size marker.

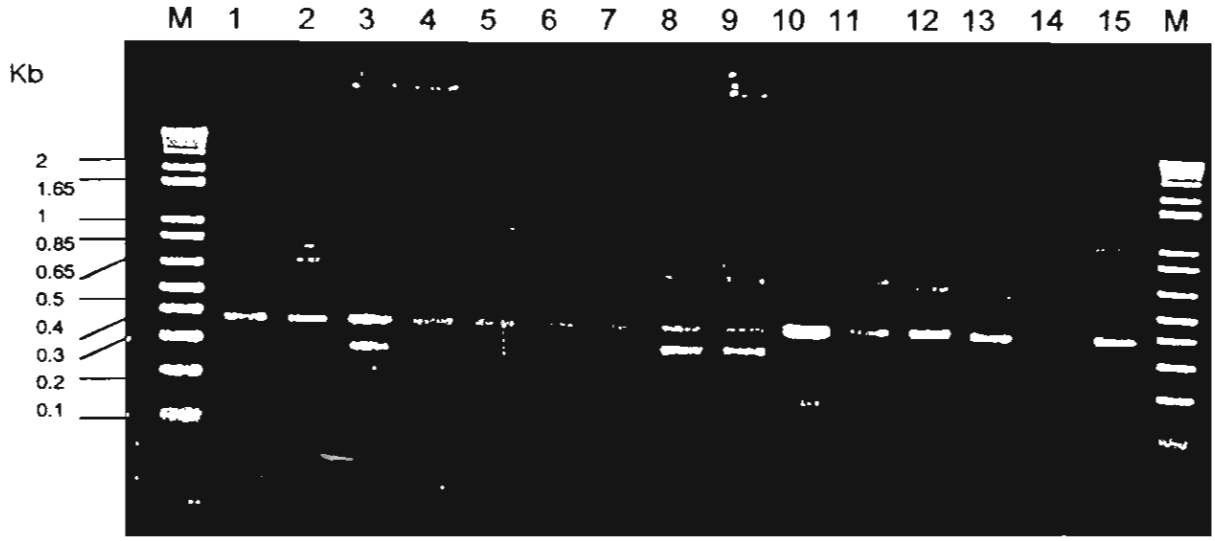

FIG 2. PCr detection of *arr* gene from *Mycobacteria* : lane 1-9, 11-13 is *M. chelonae* clinical strains, lane 14-15 is *M. fortuitum* clinical strains, lane 10 *M. smegmatis* DSM 43756 is positive control for PCR reaction and M isstandard size marker.

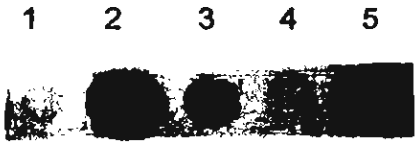

FIG 3. Dot blot hybridization of *arr* gene with *Mycobacteria*: 1 and 3 are *M. chelonae* clinical strains, 2 is *M. smegmatis* DSM 43756, 4 is *M. fortuitum*, 5 is the arr PCR product of *M. smegmatis* DSM 43756.

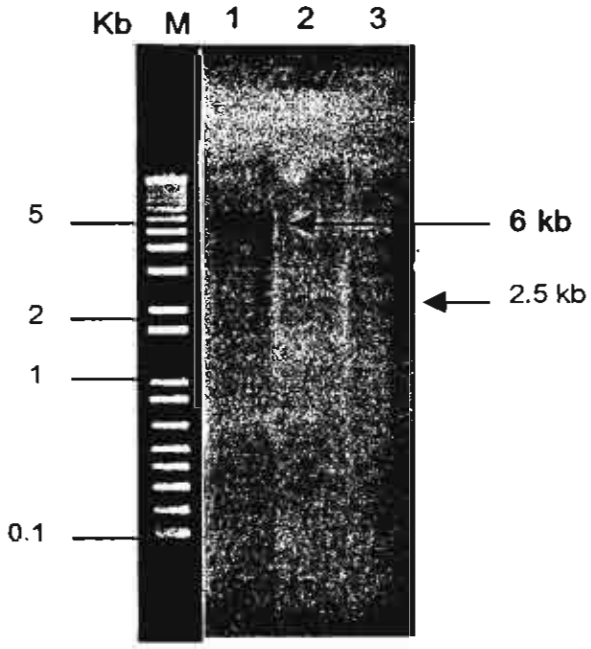

FIG 4. Southern blot hybridization of *M. smegmatis* DSM 43756 arr PCR product with EcoRI and BamHI cut DNA of lane 1: *M. smegmatis* DSM 43756, lane 2: *M. chelonae*, lane 3: *M. fortuitum*. M is standard size marker.

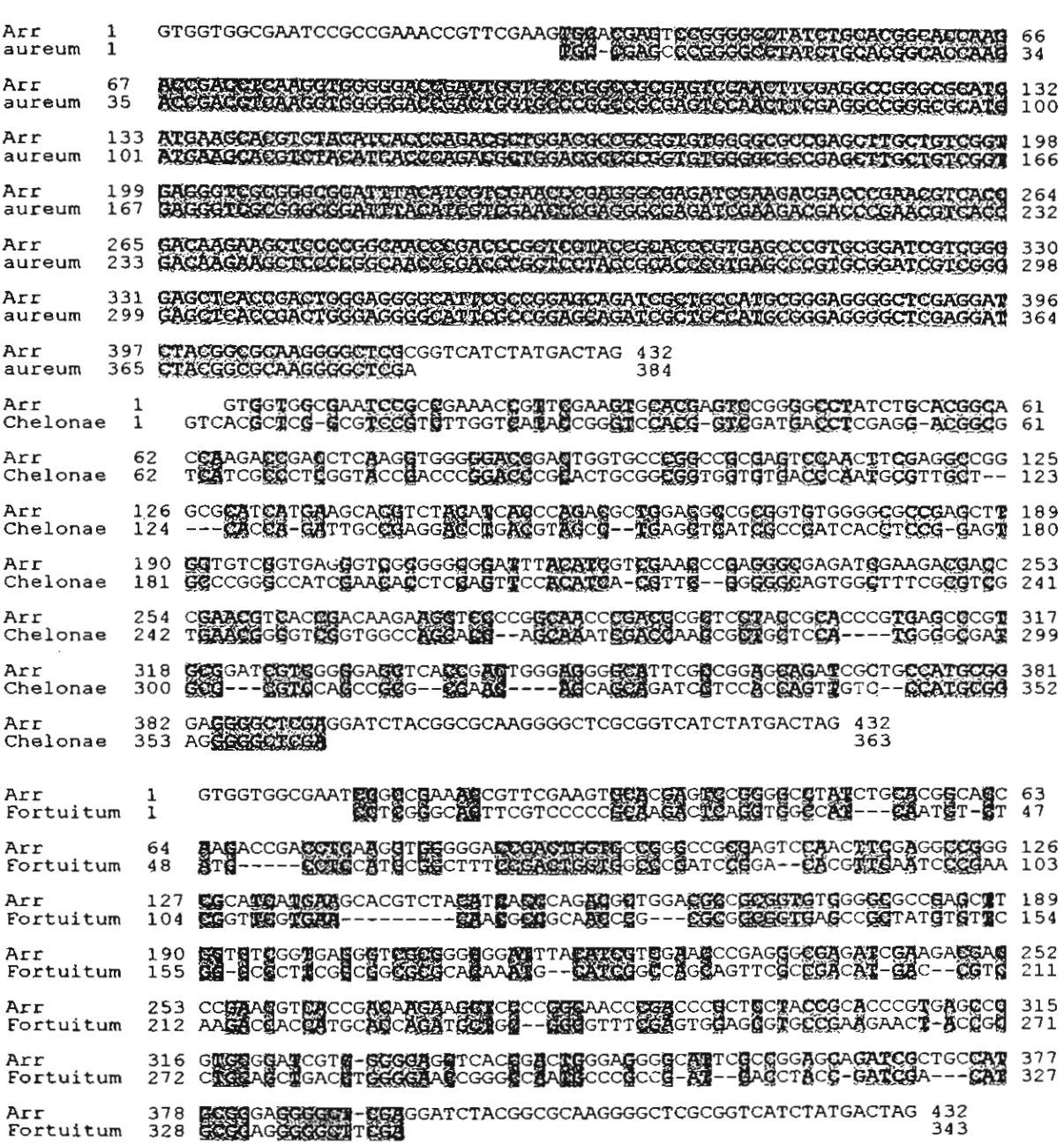

FIG 5. DNA alinement of arr gene of *Mycobacterum aurum, M. chelonae, M. fortuitum* and *M. smegmatis* DSM 43756 (*arr*). The dashes indicate gap inserted to maintain optimal alignment. Gray zones indicate identical bases.