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Research Article

INVESTIGATION OF *ACCD3* GENE OF *MYCOBACTERIUM TUBERCULOSIS* IRAQI ISOLATES

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ABSTRACT

Objective: *Mycobacterium tuberculosis*, one of the deadliest human pathogens, causes several million new infections and about 2 million fatalities annually. The cell wall of *M. tuberculosis* is endowed with a highly impermeable, complex array of diverse lipids such as mycolic acids, which bestow the bacterium with not only virulence but also resistance to host immunity and antibiotics.

Methods: Mycobacterial lipid metabolism has thus emerged as an attractive target for the design and development of novel antimycobacterial therapeutics. The first committed step in the biosynthesis of mycolic acid is the carboxylation of acetyl-CoA to malonyl-CoA which is catalyzed by acetyl-coenzyme A carboxylase carboxyl transferase beta subunit (*accD3*), a primer pairs were designed computationally and used for the amplification of *accD3* gene using conventional polymerase chain reaction (PCR) and sequencing the PCR product and analyze the results.

Results: Two sequences of the detection gene (*LprM* gene) and eight sequences of *accD3* gene under study were deposited at NCBI – GenBank database with accession numbers (LC009881, LC009880.1, LC006979, LC008196, LC009412, LC009414, LC034168, LC038020, LC041163, and LC041368) and primer pairs deposited at Probe database/NCBI with accession number Pr032816836.

Conclusion: *AccD3* gene is a good drug target in MDR *M. tuberculosis* strains.

Keywords: Tuberculosis, *AccD3*gene, Mycolic acid, Primer design, BLAST distance tree.

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INTRODUCTION

Tuberculosis (TB) is a debilitating and highly contagious disease that primarily affects the lungs. It was declared by a global health emergency in 1993 by the World Health Organization when approximately 8 million TB cases were estimated, and 1.3–1.6 million deaths occurred from the disease every year, now TB exacerbated by the spread of AIDS [1,2].

AccD3 gene, one of the virulent factors, plays a key role in the biosynthesis pathway of cell wall mycolic acid, and the product of this gene is acetyl-CoA carboxylase carboxyl transferase beta subunit enzyme which is one of the *Acc* groups. Currently, there are no examples of antibacterial *Acc* inhibitors in clinical use as antibiotics [3-5]; therefore, *accD3* represents a very good target for drugs [6].

The aim of this study is to investigate this gene in Iraqi isolates, to be used later for drug design or discovery.

METHODS

DNA extraction from *Mycobacterium tuberculosis* isolates and primer design

A total of 50 mycobacterial cultures were collected from the Institute of Chest and Respiratory Disease in Baghdad/Iraq, from May to July 2013. DNA was extracted from prediagnosed mycobacterial cultures and purified by CTAB method [7,8]. *M. tuberculosis* isolates were characterized at molecular level using *LprM* gene, and two sequences of *LprM* gene were deposited at GenBank database with accession numbers (LC009880.1 and LC009881), primer pairs were used to amplify this gene to differentiate *M. tuberculosis* from the other types of mycobacteria [9]. The results showed that 43 isolates (86%) among of 50 isolates were *M. tuberculosis*.

DNA sequences of *accD3* (1488 bp) were retrieved from public database; primers were designed for the gene at two positions, Segment I from 38 to 831 to give polymerase chain reaction (PCR) product 793 bp and Segment II from 631 to 1482 to give PCR product 851 bp. There was an overlapped sequence about 300 bp which was curated manually. The resulted sequence covered the region from 39 to 1482, these were aligned using Clustal W, and phylogenetic tree was built using NJ method, all these sequences were deposited at NCBI/nucleotide (GenBank database) with accession numbers (LC006979, LC008196, LC009412, LC009414, LC034168, LC038020, LC041163, and LC041368).

The primer sequences used in PCR experiments are deposited at Probe database/NCBI with accession number (Pr032816836) shown in Table 1.

Amplification of target gene

AccD3 gene segments (SI and SII) were amplified separately. Each PCR mixture was prepared with 25 µl of Green Master Mix ×2 (Promega), 17 µl of nuclease free water, 2 µl of each primer (F,R) at 10 µM, and 4 µl of DNA (equaling 25–250 ng). Multiple PCR programs were used until reach to the optimum program which gives a good PCR product, the thermocycling conditions for SI and SII were 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, and annealing at 59°C for 1.30 min and 72°C for 1 min, then the final extension steps at 72°C for 10 min.

PCR amplified products (10 µl) and DNA molecular-weight marker (Ladder) were electrophorized on 1% agarose gel with ethidium bromide staining to verify the size of the amplicon, the resulted PCR products were sequenced (NICEM-USA, Apparatus: Applied Biosystem).

RESULTS AND DISCUSSION

M. tuberculosis contains multiple versions of *accD* genes that encode α and β subunits of at least three distinct multifunctional acyl-CoA carboxylase complexes [10,11]. The function of a number of genes

Table 1: Primer pairs for *accD3* gene segments (used in this study)

Segment I (SI) (38–831)	Forward	GCTAGACCGGGGATCTTTCG	PCR product	793bp
	Reverse	GCCTTGATCGGTTCCTGACA		
Segment II (SII) (631–1482)	Forward	TGAGTTGCTCTATGGCGACC	PCR product	851bp
	Reverse	GACAGTCGTAGGGCGAACTC		

PCR: Polymerase chain reaction

Table 2: BLASTing results of *accD3* sequences

Strain number	E value	Identity (100%)	Max score, total score	Query cover (%)	Accession number of similarities strains and range
ALQM1	0.0	99	2710,2710	100	HG813240.1 3042536 to 3044023
ALQM2	0.0	99	2732,2732	100	CP009100.1 1006688 to 1008175
ALQM3	0.0	99	2732,2732	100	CP007299.1 1004529 to 1006016
ALQM4	0.0	99	2693,2693	100	CP007027.1 1006696 to 1008183
ALQM5	0.0	99	2473,2473	99	AP014573.1 1010903 to 1012244
ALQM6	0.0	99	2405,2405	99	CP009480.1 1003854 to 1005193
ALQM7	0.0	99	2416,2416	99	CP009480.1 1003854 to 1005194
ALQM8	0.0	99	2447,1447	98	HG813240.1 1005257 to 1006587

Table 3: The BLAST results of *accD3* protein sequences with others in Uniprot database

Strain number	E-value	Identity	Accession number of subject (protein strains)
ALQM1	0.0	100	A0A097ZPG9
ALQM2	0.0	100	A0A097ZP91
ALQM3	0.0	100	A0A0A1GNR9
ALQM4	0.0	100	A0A0A1GN94
ALQM5	0.0	99.6	A0A097ZPG9
ALQM6	0.0	97.8	A0A0A1GNR9
ALQM7	0.0	98.4	A0A0A1GNR9
ALQM8	0.0	99.6	A0A056FNG1

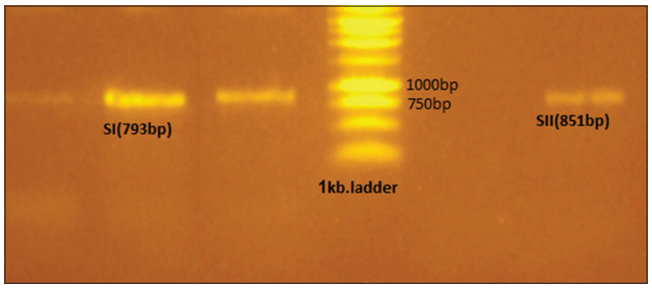

Fig. 1: Gel electrophoresis of *accD3* (SI and SII) using 1% agarose staining with ethidium bromide

involved in fatty acid and mycolic acid biosynthesis is known for their role in the survival of pathogenic *M. tuberculosis* [12-14]. A primer pairs for β subunit were designed and used in the amplification of this gene after segmentation of this big gene using conventional PCR technique, the resulted 30 samples were positive out of 43 samples. Amplified *accD3* gene (SI and SII) results are shown in Fig. 1.

The PCR product for the two segments of eight isolates was sequenced and *in silico* analyzed to prove the conformity with *accD3* gene sequences of other *M. tuberculosis* strains in the public databases, Segment I (SI) and Segment II (SII) sequences were BLASTed at expected threshold value 0.05 using BLASTn program and Nucleotide Collection (nt/nr) database. The results showed these sequences lie in *M. tuberculosis* strains with identity 98–99% as shown in Figs. 2 and 3, and the clustering results of these sequences with other *Mycobacterium* strains are shown in Figs. 4 and 5.

There was an overlap sequence with length about 300 bp between SI and SII, this was removed and merged using Mega 6 and merger tools at EMBOSS package (http://emboss.bioinformatics.nl/cgi-bin/emboss/merger) [15] to obtain the whole curated sequence. The obtained sequences of *accD3* were BLASTed with other strains in GenBank database at E. value 0.00, and the results were showed that the query sequences were matched the *accD3* sequences in the databases with query cover 100% and identity percentage 99%. Table 2 summarizes the results of BLAST program (nucleotide Blast) for the eight obtained *accD3* sequences which were designated as (ALQM1, ALQM2, ALQM3, ALQM4, ALQM5, ALQM6, ALQM7, and ALQM8).

AccD3 (*Rv0904*c) sequences (ALQM1, ALQM2, ALQM3, ALQM4, ALQM5, ALQM6, ALQM7, and ALQM8) as a group (Iraqi strains) were clustered with other strains isolated from different countries (the USA, Russia, India, Japan, India, and Colombia) and registered in NCBI and DDBJ databases. The genetic changes were between the members of phylogenetic tree was 0.02 and the query sequences distributed on different sites. Fig. 6 shows the phylogenetic tree of *accD3* nucleotide sequences, using nucleotide sequences.

accD3 sequence translated into protein sequence and the resulted protein sequences were aligned with others in the public database using Uniprot database/Blast (http://www.uniprot.org/blast/). The protein BLAST results are summarized in Table 3.

According to our knowledge, this is the 1st time to design primer pairs for *accD3*, and the results showed that the designed primer in this study might be efficient and realistic for the detection of this gene.

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Mycobacterium tuberculosis strain ZMC13-264, complete genome	1363	1363	98%	0.0	100% CP009100.1
Mycobacterium tuberculosis strain 0A029DS genome	1363	1363	98%	0.0	100% CP008981.1
Mycobacterium tuberculosis strain 0A033DS genome	1363	1363	98%	0.0	100% CP008980.1
Mycobacterium tuberculosis strain 0A087DS genome	1363	1363	98%	0.0	100% CP008978.1
Mycobacterium tuberculosis strain 0A092DS genome	1363	1363	98%	0.0	100% CP008977.1
Mycobacterium tuberculosis strain 0A093DS genome	1363	1363	98%	0.0	100% CP008976.1
Mycobacterium tuberculosis strain 0A094DS genome	1363	1363	98%	0.0	100% CP008975.1
Mycobacterium tuberculosis strain 0A115DS genome	1363	1363	98%	0.0	100% CP008974.1
Mycobacterium tuberculosis strain 0A117DS genome	1363	1363	98%	0.0	100% CP008973.1
Mycobacterium tuberculosis strain 0B070XDR genome	1363	1363	98%	0.0	100% CP008970.1
Mycobacterium tuberculosis strain 0B123ND genome	1363	1363	98%	0.0	100% CP008968.1
Mycobacterium tuberculosis strain 0B222DS genome	1363	1363	98%	0.0	100% CP008965.1
Mycobacterium tuberculosis strain 0B228DS genome	1363	1363	98%	0.0	100% CP008964.1
Mycobacterium tuberculosis strain 0B229DS genome	1363	1363	98%	0.0	100% CP008963.1
Mycobacterium tuberculosis strain 0B235DS genome	1363	1363	98%	0.0	100% CP008962.1
Mycobacterium tuberculosis strain 0B259XDR genome	1363	1363	98%	0.0	100% CP008961.1
Mycobacterium tuberculosis strain 0B329XDR genome	1363	1363	98%	0.0	100% CP008960.1
Mycobacterium tuberculosis strain 6A024XDR genome	1363	1363	98%	0.0	100% CP008959.1

Fig. 2: Alignment results of SI sequence against public database

Mycobacterium tuberculosis strain 0A005DS genome	1367	1367	97%	0.0	97% CP008983.1
Mycobacterium tuberculosis strain 0A029DS genome	1367	1367	97%	0.0	97% CP008981.1
Mycobacterium tuberculosis strain 0A033DS genome	1367	1367	97%	0.0	97% CP008980.1
Mycobacterium tuberculosis strain 0A087DS genome	1367	1367	97%	0.0	97% CP008978.1
Mycobacterium tuberculosis strain 0A092DS genome	1367	1367	97%	0.0	97% CP008977.1
Mycobacterium tuberculosis strain 0A093DS genome	1367	1367	97%	0.0	97% CP008976.1
Mycobacterium tuberculosis strain 0A094DS genome	1367	1367	97%	0.0	97% CP008975.1
Mycobacterium tuberculosis strain 0A115DS genome	1367	1367	97%	0.0	97% CP008974.1
Mycobacterium tuberculosis strain 0A117DS genome	1367	1367	97%	0.0	97% CP008973.1
Mycobacterium tuberculosis strain 0B026XDR genome	1367	1367	97%	0.0	97% CP008972.1
Mycobacterium tuberculosis strain 0B070XDR genome	1367	1367	97%	0.0	97% CP008970.1
Mycobacterium tuberculosis strain 0B076XDR genome	1367	1367	97%	0.0	97% CP008969.1
Mycobacterium tuberculosis strain 0B123ND genome	1367	1367	97%	0.0	97% CP008968.1
Mycobacterium tuberculosis strain 0B169XDR genome	1367	1367	97%	0.0	97% CP008967.1
Mycobacterium tuberculosis strain 0B218DS genome	1367	1367	97%	0.0	97% CP008966.1
Mycobacterium tuberculosis strain 0B222DS genome	1367	1367	97%	0.0	97% CP008965.1
Mycobacterium tuberculosis strain 0B228DS genome	1367	1367	97%	0.0	97% CP008964.1
Mycobacterium tuberculosis strain 0B229DS genome	1367	1367	97%	0.0	97% CP008963.1

Fig. 3: Alignment results of SII against bacterial strains in public database

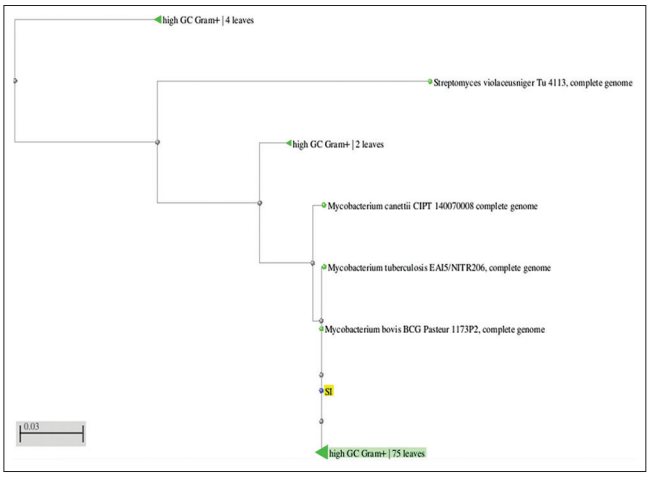

Fig. 4: BLAST distance tree of SI with others in public database

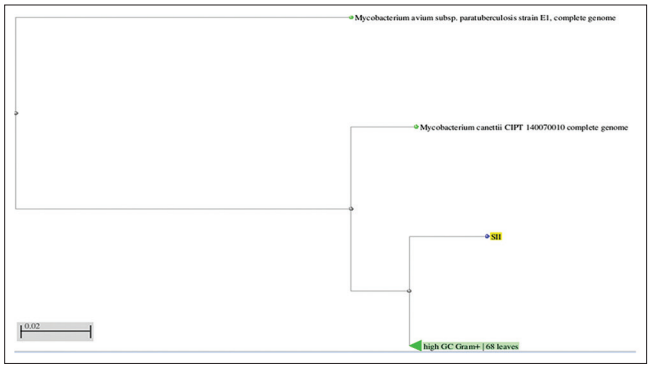

Fig. 5: BLAST distance tree for SII with other *Mycobacterium strains*

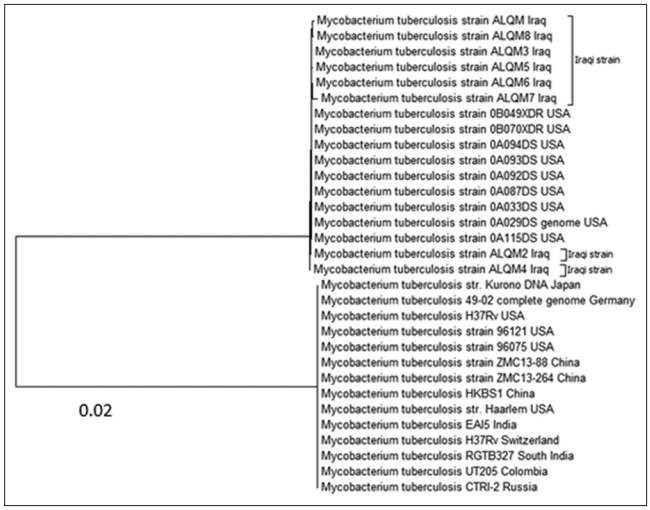

Fig. 6: Phylogenetic relationship of *accD3* nucleotide sequences

AUTHORS' CONTRIBUTIONS

Asra'a A. Abdul-Jalil and Zahra M. Alkhafaji achieved bioinformatics analysis and primers design. Mushtak T. Al-Ouqaili helped us in the performing of molecular experiments.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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