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# Sequence Homology Studies of Phospholipase A<sub>2</sub>-like Gene from Bloodstream form of *Trypanosoma brucei*

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#### Authors' contributions

This work was carried out in collaboration between all authors. Author AJN designed the study and wrote the protocol. Author IYL managed the analyses of the study and wrote the first draft of the manuscript. Authors HMI and IAU performed the Bioinformatics analysis and the literature searches. All authors read and approved the final manuscript.

#### Article Information

DOI: 10.9734/ BBJ/2015/13971 <u>Editor(s):</u> (1) Laura Pastorino, Dept. Informatics, Bioengineering, Robotics and Systems Engineering (DIBRIS), University of Genoa, Italy. (2) Chung-Jen Chiang, Department of medical laboratory Science and Biotechnology, China Medical University, Taiwan. <u>Reviewers</u>. (1) Anonymous, University of New Mexico and New Mexico VA Health Care System, Albuquerque, USA. (2) Anonymous, Federal University of Rio de Janeiro, Brazil. (3) Anonymous, University Brunei Darussalam, Brunei. (5) Anonymous, Simon Bolivar University, Venezuela. Complete Peer review History: <u>http://www.sciencedomain.org/review-history.php?iid=803&id=11&aid=7566</u>

**Original Research Article** 

Received 12<sup>th</sup> September 2014 Accepted 28<sup>th</sup> November 2014 Published 31<sup>st</sup> December 2014

# ABSTRACT

**Aim:** This work focused on the sequence homology studies of the enzyme, phospholipase A2 (PLA<sub>2</sub>), in *Trypanosoma brucei* obtained from the blood of bull in Federe, Plateau State, Nigeria, West Africa

**Place and Duration of Study:** Department of Biochemistry, University of Jos, Nigeria; Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria, Department of Biotechnology, NVRI, Vom, Nigeria; between June 2009 and September 2011.

**Methodology:** *T. brucei* grown in rats were harvested and separated using diethyl amino ethyl (DEAE) cellulose chromatography. From the parasites' genomic DNA the PLA<sub>2</sub>-like gene was amplified using consensus primers. The amplicon was cloned unto pMal-2cE vector and confirmed using direct PCR and restriction enzyme analyses. The PLA<sub>2</sub> gene and translated protein



sequences were studied using National Center for Biotechnology Information (NCBI) Conserved Domain Search Tool and Conserved Domain Architectural Retrieval Tool

**Results:** Analyses of the 1344bp gene sequence using bioinformatics tools showed that it is very closely related to PLA<sub>2</sub> sequences of *T. brucei* (TREU 927) and *T. b. gambiense*. Motifs that are unique to PLA<sub>2</sub> (FSHGL) and lipases (GHSFG) were found to be present in the query sequence. The domains present in the studied sequence agreed closely with those of the human platelet activating factor acetyl hydrolase (PAF-AH). There was also a good sequence resemblance with PLA<sub>2</sub>s from *T. cruzi, Metarhizium amisop, Metarphizium acridu* and PAF-AH in terms of architecture.

**Conclusion:** The  $PLA_2$ -like gene isolated from the blood stream form of *Trypanosoma brucei* and studied was found to posses the domains and motifs unique to  $PLA_2$ s and lipases and so homology was established among the proteins.

Keywords: Trypanosoma brucei; Phospholipase A<sub>2</sub>; gene; motif; domain.

#### 1. INTRODUCTION

Phospholipase  $A_2$  (3.1.1.4) comprises of a diverse family of enzymes that hydrolyze glycerophoshospholipids at the sn-2 position giving free fatty rise to acids and lysophospholipids. The membership of the PLA<sub>2</sub> super family is ever expanding [1]. The enzymes have been classed into 15 groups [2]. These groups were regrouped into two - those utilizing a catalytic histidine and those using a catalytic serine. All the members of the PLA<sub>2</sub> super family carry a consensus sequence GXSXG which is common to many other lipases [3] despite some differences that are the basis for the various patterns of classification. The enzymes are classed based on their source, amino acid sequence, chain length and disulphide bond patterns [4] as well as based on the guiding information that the enzyme must catalyze the hydrolysis of the sn-2 ester bond of a natural phospholipid substrate. In addition, the enzyme must have complete protein sequence of the mature protein established: have homologous enzymes distinguished and the spliced variant established within subgroups; have the sequence homology and catalytic activity established in order to be classed along with others [1].

The physiological role of PLA<sub>2</sub> gene expressed as PLA<sub>2</sub> protein includes the hydrolysis of phospholipids vielding free fatty acids (arachidonic acid and oleic acid) and lysophospholipid. The fatty acids are important stores of energy. Arachidonic acid is an important metabolic intermediate for producing eicosanoids, which are regulatory factors implicated in a wide range of physiological and pathological states serve as potent mediators of inflammation and signal transduction [5]. The other product, lysophospholipid, is important in

cell signaling, phospholipid remodeling and membrane perturbation [3]. This enzyme is also reported to regulate the entry of calcium ions into *Trypanosoma brucei* and help the parasite modulate the host-parasite interaction [6] thereby implicating PLA<sub>2</sub> in the pathogenesis of the *T*. *brucei* The PLA<sub>2</sub> family has become a major drug target for many different diseases [7,8].

PLA<sub>2</sub> has been reported in different species of trypanosomes to various degrees. Some reports show that phospholipase A<sub>2</sub> has been identified to have orthologs in T. brucei, T. cruzi, and humans and the Leishmania PLA<sub>2</sub>/PAF-AH (LmjF.35.3020) contains a predicted N-terminal signal peptide sequence and transmembrane domain and a predicted lipase/platelet-activating factor acetylhydrolase sequence [9]. This group of enzymes has been isolated from various sources such as animal toxins like snake venom [10]; insect venom [9] and mammalian organs [11,12]. Some other reports show that the enzyme has also been isolated and purified to electrophoretic homogeneity in T. congolense [13]; that activity and kinetics of  $PLA_2$  from T. brucei gambiense and T. brucei have been detected and studied [14]. The Trypanosoma brucei (Tb09.211.3650) Gene Data Base on TritrypDB hosted by the Sanger Institute, revealed a protein encoding gene was present having a product described as phospholipase A<sub>2</sub>like protein, putative. However, reports on PLA<sub>2</sub> in T. brucei distributed around the endemic regions are quite scarce. Equally, the PLA<sub>2</sub> from Trypanosoma species has not found inclusion in the several classes of the enzymes reported in literature possibly due to the scarcity of literature on its studies. Therefore, this work was designed to study the gene sequence of PLA<sub>2</sub> obtained from T. brucei in Nigeria, West Africa, for the first time, in comparison with those already

characterized. This may contribute in the understanding of the nature of the enzyme and so in subsequent classification. As part of the swollen effort, Phospholipase A2-like gene from blood stream form Trypanosoma brucei brucei had

been reported to conserve domains and motifs peculiar to characterized PLA<sub>2</sub>s and lipases [15]. Literatures have revealed that genome sequence analysis have helped in the rcharacterization of a PLA<sub>2</sub> cDNA from Arabidopsis thaliana [16] genes encoding heterodimeric phospholipases A<sub>2</sub> from the scorpion Anuroctonus phaiodactylushav [17] sequences and structural organization of phospholipase A<sub>2</sub> genes from Vipera aspis aspis, V. aspis zinnikeri and Vipera berus berus venom [18] plasmid pLA1 present in Ν pentaromativorans US6-1 [19]; this approach has therefore found application in Metagenomics [20].

# 2. MATERIALS AND METHODS

# 2.1 Reagents and Equipment

Chemicals used were of analytical grade and purchased from Sigma and Pharmacia Fine chemicals. DNA extraction kit was purchased from Bio Basic Inc. Markham Ontario, Canada. Tag polymerase and High Fidelity Polymerase Enzyme Mix were bought from Promega, USA Fermentas, respectively. High and Pure polymerase chain reaction (PCR) Clean-Up Kit used to purify PCR products was purchased from Fermentas and 100 bp DNA molecular size marker was purchased from Roche, Mannheim Germany. Products from Fermentas and oligonucleotide primers were supplied and synthesized by Inqaba biotec Industry®, Pretoria South Africa. GeneAmp PCR System9700 used for amplification was obtained from Applied the Biosystems, Indonesia and Gel Documentation System was obtained from Synegene® Inc. Indonesia. Sequencing analyses were performed by Ingaba Biotec Industries®, Pretoria South Africa.

# 2.2 Parasites Isolation

An isolate of T. b. brucei from cows (Federe isolates) were obtained from the Parasitology Department, NITR, Vom. Adult albino rats were infected through intra peritoneal route with 0.2ml Trypanosoma brucei infected blood diluted with 2ml phosphate saline glucose (PSG) buffer pH 8.0 to give cell density of  $\approx 1 \times 10^6$  cells/ml. Parasitemia was monitored by wet smear via tail snip. At peak parasitemia ( $\approx 1 \times 10^8$  cells/ml), the rats were euthanized and blood collected. Parasites were purified on DEAE-cellulose (prewhatman DE-52-Pharmacia Fine Chemicals) as previously described [21].

# 2.3 DNA Isolation

Genomic DNA was extracted from 200µl (≈ 1 x 10<sup>8</sup> cells/ml) of isolated *T.b. brucei* suspended in PSG buffer (0.6g NaH<sub>2</sub>PO<sub>4</sub>; 0.71g Na<sub>2</sub>HPO<sub>4</sub> and 14.61g NaCl in 450ml distilled water adjusted to pH 8.0 with orthophosphoric acid) using ZR Genomic DNA Tissue Minipreps Kit (Zymo Research) according to the manufacturer's instructions. Briefly, cell lysis was with 500 µl lysis buffer (which consisted of 10 mM phosphate buffer containing and protease inhibitor) and proteinase K (3 µl of 10mg/ml) incubated at 55° C for 30 minutes. DNA was precipitated with 260 µl absolute ethanol. Precipitated DNA was captured in EZ-10 column by centrifuging at 12,000 x g for 1 minute. The flow through was discarded while the EZ-10 column placed in a fresh vial and centrifuged again at 12,000 x g for 1minute to remove residual wash buffer. DNA was eluted into 1.5 ml microcentrifuge tubes with 50 µl elution buffer after incubation at 50°C for 2 minutes by centrifuging at 14,000 x g for 1 minute.

### 2.4 PCR Amplification of Phospholipase A<sub>2</sub> Like Gene

Phospholipase A<sub>2</sub> like gene was detected and amplified by Polymerase Chain Reaction (PCR) with primers designed based on the gene sequence of PLA<sub>2</sub> like gene in the GeneBank Data Base (Tb 09.211.3650, Phospholipase A2like protein, putative, T. brucei, chr 9). The primer designed was done in Ingaba Biotech Industry, Pretoria, South Africa. The primers used were as follows: Sense primer 5'-ATGGTAACGTGGGC GCTGAA GTAT- 3' carrying BamHI site and Antisense primer 5'-CTAACACGTTGAACACACTTC GGTA-3'carrying Pstl site. High Fidelity Taq DNA Polymerase Enzyme kit (Fermentas) was used to amplify the gene from the genomic DNA according to the manufacturer's instructions. The optimum reaction mix in 50 µl volume was as follows: nuclease free water (37.6µl); 10 x PCR buffer (5.0 µl); dNTP mix (1.0 µl); each primer (1.0 µl); High Fidelity Taq enzyme mix (0.4 µl); Genomic DNA (5.0 µl of 50µg DNA/ml). The thermal cycling was carried out with the following process profile: initial denaturation at 94°C for 2minutes, elongation 94°C for 30 seconds, 56°C for 30 seconds, 68°C for 2 Longdet et al.; BBJ, 5(3): 156-165, 2015; Article no.BBJ.2015.015

minutes running for 30 cycles, and final extension at  $68^{\circ}$ C for 10 minutes; then ending/waiting at  $4^{\circ}$ C for  $\infty$ . Ten microliters (10 µl) of the product was separated on 1.0% agarose gel to check the success of the process and the results documented using Gel Documentation System (Synegene®).

## 2.5 Cloning and Sequencing

The PLA<sub>2</sub> gene amplified by PCR from T. b. brucei was purified using High Pure PCR Product Clean-Up Kit (Fermentas) and ligated into pMalc2E vector in a 20 µl ligation reaction. The ligation reaction mixture was incubated at 16°C over night and subsequently used for transformation. The recombinant plasmid was designated pMal-PLA<sub>2</sub> and transformed into *coli* DH5α Escherichia competent cells. Transformants were placed on LB agar containing 100µg/ml ampicillin. PCR Cloning Kit (Fermentas) was used to clone the purified PLA<sub>2</sub> from T. b. brucei. Colonies were randomly selected for screening of positive clones by PCR and restriction endonucleases digestion of plasmids using BamHI and Pst I. The sequence was submitted to the Gen Bank Data Base.

### 2.6 Transformation of Clone into *E. coli* (BL-21 (DE3)) and Expression

One micro liter of the ligated DNA construct was transformed into E. coli expression grade BL-21 (DE3) competent cells (Lucigen) according to the manufacturers' instruction. The colonies of the BL-21 (DE3) cells were picked from the agar plates and inoculated in 30ml broth medium (SOC plus 100µl/ml ampicillin) and incubated for 3 hours at 37°C. The cells were then harvested by centrifugation at 5000g for 15 minutes at 4°C. The medium was discarded from control and induced cells. The cell pellets were re-suspended in 5 ml lysis buffer and sonicated for short pulses of 10 seconds for 4 times on ice. The lysates were centrifuged at 10, 000g for 20 minutes at 4°C. The supernatant which had the protein was collected. The pellet was re-suspended in 5 ml lysis buffer. All supernatants were then analyzed on 10 % SDS-PAGE.

#### 2.7 Bioinformatics Sequence Analysis

The Finch TV® programmes (GeoPiza) was used to analyze the PLA<sub>2</sub> gene while NCBI BLAST programmes such as NCBI Conserved Domain Search Tool (CDD) and NCBI

Conserved Domain Architectural Retrieval Tool (CDART) were used to study the PLA<sub>2</sub> gene and translated protein sequences in order to establish the possibility of homology or similarity.

# 3. RESULTS

The PCR amplification gave a 1.3kb PLA<sub>2</sub> like band from the gene from genomic DNA of Trypanosoma brucei. The amplicon cloned unto pMal-c2E vector between the restriction sites of BamH1 and Pst1 vielded pMal-PLA<sub>2</sub> of about 7.9kb which is approximately the size of the pMal-c2E vector (6.6kb) and PLA<sub>2</sub> (1.3kb) put together. Direct PCR analysis done to confirm the quality of the insert DNA gave amplicon of about 1.3kb which was similar in size with the PLA<sub>2</sub> like gene amplified from the genome DNA of bloodstream form T. b. brucei. The digestion of the purified pMal-PLA<sub>2</sub> clone and separation on agarose gel electrophoresis equally revealed the pMal-2cE vector with about 6.6kb as well as the PLA<sub>2</sub> like gene (1.3kb). These confirmed the cloning of the PLA<sub>2</sub> like gene into pMal-2cE to pMal-PLA<sub>2</sub> clone. The heterologous form expression of T. brucei PLA<sub>2</sub> in pMal-c2E plasmid recorded a success in the transformation process of the competent E. coli cells. On the other hand, the heterologous expression of the recombinant PLA<sub>2</sub> in BL-21 (DE3) competent E. coli cells was not successful. The corresponding fractions obtained from the colonies were resolved on SDS-PAGE. This put a limit to biochemical characterization of protein in order to confirm the finding from bioinformatics analysis.

The gene sequence with 1, 344bp nucleotides primary sequence obtained has been assigned the accession number JN603736. The BLASTN Alignment view programme compared the PLA<sub>2</sub> like gene sequence with gene sequences were from *T. brucei* TREU927 PLA<sub>2</sub> like protein (Gene ID: Tb 3661014 Tb 09.211, 3650; XM 822413.1) and *T. brucei* gambiense (DAL 972, FN 554972.1).

The detail NCBI BLASTN alignment view (Fig. 1) showed that no deletion occurred in the PLA<sub>2</sub> like gene (query) except eight substitutions distributed at positions 51, 61, 178, 282, 530, 810, 888 and 970 as compared with the two gene sequences in the Gene Bank DB. The substitutions in positions 51, 61, 282, 530, and 888 do not change the amino acid in those respective positions because the new codons still coded for the same amino acids. On the other hand, the substitutions in positions 178, 810 and

970 caused changes in amino acids in those positions i.e. at position 78 [TTG changed to TTC]: TTG codes for Leucine while TTC codes for Phenylalanine; at position 810 [AGT changed to ATT]: AGT codes for Serine while ATT codes Isoleucine and at position 970 [GTA changed to CTA]: GTA codes Valine while CTA codes for Leucine.

The parasite Genomes WU-BLAST2 analysis of the sequence gave 99% identity and similarity to the *T. brucei* (TREU 927) PLA<sub>2</sub> like sequence from partial mRNA, chromosome 9 and *T. brucei* gambiense DAL 972 Chr 9, complete sequence PLA<sub>2</sub> (Table 1). The result also revealed that there was 100% coverage, no gaps, 99% identity and zero E – values in each comparison. The translated PLA<sub>2</sub> primary structure of 447 amino acids was compared with GeneBank PLA<sub>2</sub> sequence Databases in NCBI using blastx established homology with those of other putative PLA<sub>2</sub> proteins from *T. brucei* (TREU927), *T. b gambiense* (DAL972), *T. cruzi*, *Leishmannia major* and characterized human Platelet – Activating Factor Acetyl Hydrolase (Fig. 2). The alignment showed that the protein carries a conserved *GHSFG* lipase motif and an *FSHGL* motif peculiar to PLA<sub>2</sub>s which are also conserved in the query. These significantly elaborated the query sequence similarity with the PLA<sub>2</sub> family.

Query         1         ATGGTAACGTGGGCGCTGAAGTATTTTGTTCGCGTAGTCGATGGTCGACAGAAGCATTC         60           DAL972:1656744	
TREU927:       61	
DAL972:1656744	
Query         61         CTAATTTGGCCCACACGGCCACTTTTTGACTATGCCACCTCATTGCATTGTGTTCCCATA         120           TREU927         61         C         120           DAL972:1656804         T         1656           Query         121         AGCGGCACATTTATTACTTCGGTTCTGCTCTGCTCTCCCACTTTTGTGGTCT         180           TREU927         121         C         180         1656           Query         121         C         1656         1656           Query         121         C         180         1656           Query         241         CCACTATTGAAACCCATTGGCGGTCGCTATAGCGTGGGCCTCGTGCATATGAACGGCTGC         300           TREU927         241	303
TREU927       61       C       120         DAL972:1656804       T       1656         Query       121       AGCGGCACATTTATTACTTCGGTTCTGCTCTGCTCTACGGGTTCCCACTTTTGTGGTCT       180         TREU927       121	
DAL972:1656804         T.         1656           Query         121         AGCGGCACATTTATTACTTCGGTTCTGCTCTGCTCTACGGGTTCCCACTTTTGTGGTCT         180           TREU927         121         C.         180           DAL972:1656864         G.         1656           Query         241         CCACTATTGAAACCCATTGGCGGTCGCTATAGCGTGGGCCTCGTGCATATGAACGGCTGC         300           TREU927         241         T.         300	
Query         121         AGCGGCACATTTATTACTTCGGTTCTGCTCTGCTCTACGGGTTCCCACTTTTGTGGTCT         180           TREU927         121	863
TREU927         121         C.         180           DAL972:1656864	
DAL972:1656864	
Query         241         CCACTATTGAAACCCATTGGCGGTCGCTATAGCGTGGGCCTCGTGCATATGAACGGCTGC         300           TREU927         241	923
TREU927 241	
D&TUT7+1656U8.4 C 1657	n42
Overy 481 δλασσαστασσασσαστασταδικά δια	545
IKEU927 401	
DAL972:1657224	283
Query 781 AAGGACTTTTGGACAACTTTGGGCTACAGTAATTCAGATATTGACAAGTTTCTTAGCAAA 840	
TREU927 781	
DAL972:1657524	583
Query 841 CCGTTGCAGGTACATCTTGCGGGTCATTCATTTGGCGGTGCCACTGTACTCGCGGCTGCA 900	
TREU927 841	
DAL972:1657584	643
Query 961 CCATGGATGGTACCAATACAAAATGAACATTTTTGCAACCCGCTTTCTGATGGCCGTAAA 1020	
TREU927 961	
DAL972:1657704	763
Query 1321ACCGAAGTGTGTTCAACGTGTTAG 1344	
TREU927 1321ACCGAAGTGTGTTCAACGTGTTAG 1344	
DAL972:1658064ACCGAAGTGTGTTCAACGTGTTTAG 1658087	

Fig. 1. NCBI BLASTN Alignment View of PLA<sub>2</sub> like gene sequence with *T. brucei* TREU927 PLA<sub>2</sub> like protein from partial mRNA length = 1344(Gene ID: Tb 3661014 Tb09.211.3650; XM 822413.1) and *T.brucei* gambiense DAL972, chromosome 9, complete sequence length = 2,160,261 (FN554972.1)

# Table 1. Comparison of PLA<sub>2</sub>-like gene sequence with those of two sequences in data base (DB) using NCBI and Parasite Genomes Washington University Basic Local Alignment Search Tool 2 (WU-BLAST2)

DB- AN	Source	Мах	Total	Length(bp)	Query	Identity	Gaps	E.value
		score	score		coverage (%)	(%)	(%)	
FN554972.1	T. brucei	2640	2640	2,160,261	100	99	0	0
	aambiense							
XM8412121	Thrucei	2640	2640	1 344	100	99	0	0
ANIO41212.1		2040	2040	1,344	100	33	0	0
	TREU927							
Each source pa	rasite is identified	d by its dat	a base acce	ession number (l	DB-AN) and the s	equence le	ngth give	n in base
			p	oairs (bp)				
Query	TAMVAV	GETISGUI	FLVSPLPL	LKPTCCRYSVCL	WHMNGCRSOSTP	PVAVEYPT	MVPRK	120
TREU927	TAMVAV	GFIISGVI	FLVSPLPL	LKPICGRYSVGI	WHMNGCRSQSIP	PVAVEYPT	MVP EK	120
TbgPLAZ	TAMVAV	GFIISGVI	FLVSPLPL	LKPIGGRYSVGI	WHMNGCRSQSIP	PVAVF YP TI	MVP EK	120
TcPLA2	LPIIPL	GIIGAAVI	FILLPUPI	L EPV CCHYHVC 1	WHVHSPHSQ TMP	PLAVYYP TI	TPRR	107
LmPLA2	IMMTCV	GSLVTCAU	FYVQPLQC	FSPLCCSFNVCT	REVCGERGAME	PVTIVYPT.	ASCTPR	96
HSPAFAH	AWVNKI	QVLMAAAS	FGQTKIPN	GNGPYSVGC	TDLMFDHTNKGT	FLRLYYPS	JDN. DL	9Z
Consensus	• • • • • • •		<b>X</b>	g vg.				
Query	KGL PYV	PF CDD PF I	.PCVAAYAN	V PFFFIPDFSFV	TI SASPNAU PAA	LLNQYERV	PPIVV	180
TREU927	KGL PYV	PF GDD RF I	RCVAAYAN	VPFFFIPDFSFV	RISASPNAV PAA	LLNQYERV	PPIVV	180
TbgPLA2	KGLPYV	PFGDDRFI	RCVAAYAN	VPFFFIRDFSFV	TISASRNAV PAA	LLNQYERV	PPIVV	180
TCPLAZ	RGIQYI	PFNDVPF	ISCLSSRAC	VPLYLMRDFLFU	PLRATEGARPIP	LINSSCIP	LPVII	167
LMPLAZ W-DAVAU	DTLATE	NEVEN PR	NGLASISK CL SVRLCT	WPIRLVEDLULI UNIMONITIDII	CONTTO MEMOR	LFQRDGAP	VD11707	149
Consensus	5					1	.p	142
							-	
Query	FSHGLA	GYHLFYSC	FALDLAAR	GAIVICLOHODN	SASFMEDS	SCRESE	7P LKDYG	234
TheO327	POHOLA	CULL PYCC	FRED LAAN	CATUTCICHCDN	SASPIRUS	COURCES A	THE PIC	234
TOGPLA2	FSHGLY	CYHPLYS I	LOADLASP	CARRYTCHCDN	ISASPIRUS	EPGCHE	FFLOOD	201
LmPLA2	FSHGLG	GF PHLYS 1	LLMDLAVR	GAVVFALSHMDG	SAAFCEDA	ERETRI	PLNTOWC	170
HSPAFAH	FSHGLG	AFRTLYSA	IGIDLASH	GFIVAAVEHPDF	SASATYYFRDOS.	AABIGDKS	JLYL PHO	209
Consensus	fshgl.	ys.	dla	gvh.d.	sa.a			
0		A TO TR A COT 1	ODUC TID C		THE TOTAL OF THE TOTAL OF THE			201
TDELI927	WEVP	ARRAQVA ADVAOUA	ODUS WIDC	TLOPLT WET	WHTTLCY THEOT	OVFISED L	UNI.	291
ThePLA2	MEUP	APRACUA	ORVSKURG	TLOBIT KKT	FUTTLEYINSDU	DEFLSEPL	O VHL	291
TcPLA2	WDSP.	.VCEBAL	ORVMETER	TLKRLS EKE	FWKELGFAD LDA	EOYLROSP	R. VHL	2.58
LmPLA2	WTSE.	DRAPQLE	VRIRETLN	TIKRIRSGE	LLLALCYDKETVI	DKYIEKEP	R. IHL	227
H≤PAFAH	EEETHI	RNEQ VI	QRAKECSQ	ALSLILDIDHGH	<b>PVKNADLKFDME</b>	QLKDSI.D	REKIAV	266
Consensus	5		.re					
Query	AGHSFG	GATVLAAA	LERNONPVI	KGVSVKSVYTFD	PWMVPIONEHFCI	NP LSD GRK	SYTVPT	351
TREU927	AGHSFG	GATVLAAA	LEKNONPVI	KGVSVKSVYTFD	PWMVPIONEHFCI	NPLSDGRR	SYTVPT	351
TEGPLAZ	AGHSFG	GATVLAAA	LEKNONPVI	KGVSVKSVYTFD	PWMVPIQNEHFCI	NP LSD GRK	SYTVPT	351
TCPLA2	SCHSFG	GATALVAS	MQEEQESK	PNENPVQSVI	VFDPWHKPLQNE	LFLKPIEE	GRN R	347
LmPLA2	LGHSFG	GATCLAAA	LADTQAAS	ERGGVSSIASTV	VYDPWHI PLQKTI	HFYDRLTD	RKQPVH	287
HSPAFAH	IGHSFG	GATVIQTI	SEDQRFR.	. CGIALDAWMFP	LCDEVYSPIPQP	LFFINSEY	FQYPAN	324
Consensus	⊧ .gh≲fg	gat						
Query	TNHLSL	VDVSVLSI	WMHGNI	WATVSP RVO	TMEWCNALL REAL	KONTEVCS	тс	447
TREU927	TNHLSL	VDVSVLSI	WHIGNI	WATVSPRVQ	IMEWCNALL RFAI	RONTEVCS	тс	447
TbgPLA2	TNHLSL	VDVSVLSI	WHIGNI	WATVSPRVQ	IMEWCNALL RFAI	RONTEVCS	тс	447
TcPLA2	TNHEKT	CSYSVSDV	VLLSPVIH	GCKNSILSPRVQ	IMEWSNTCLRFI	KEHAMYTS:	RTSAR.	455
LmPLA2	TGHRDY	RGLTCTD	SLFSPVLY	RAAYMTASP RCC	IVAFAAETMRFI	EKVSGPLP	LDTKLL	456
MSPARAH	UKHLSL	NF LGLHKI	T LOUDOLI	BODD EN LIFGTN	TWITNGHTUPON:	SSGIBKYN		435
consensus	•n			p	******			

# Fig. 2. Amino acid sequence alignment of PLA<sub>2</sub>-like protein with PLA<sub>2</sub> from *T. brucei* (TRUE927), *T. b. gambiense* (TbgPLA<sub>2</sub>), *T. congolense* (TcPLA<sub>2</sub>), *Leishmania major* (LmPLA<sub>2</sub>) and PAF-AH. Conserved motifs and lipase consensus motif were marked with red colour

The NCBI conserved Domain search Tool analysis of the protein sequence predicted the Conserved domains (Fig. 3). The result showed that the PLA<sub>2</sub> translated protein sequence which had 447 amino acid residues was mapped to PAF-AH super family, a sub-family of PLA<sub>2</sub> super family. The conserved domains were marked in red ink. Equally predicted was the Conserved Domain Architecture of the protein sequence using the Conserved Domain Architecture Retrieval Tool (CDART).

The results (Fig. 4) showed the graphic view of conserved domains architecture on PLA<sub>2</sub> like protein. Proteins with similar architectures were PLA<sub>2</sub> from *T. cruzi*, *Metarhizium amisop*, *Metarphizium acridu* and PAF-AH.

#### 4. DISCUSSION

The gene sequence (1,344 bp) presented and analyzed using some Bioinformatics Tools revealed some interesting high lights. The

revealed 99% identity and similarity between the PLA<sub>2</sub> – like gene from *T. b. brucei* and that of *T.* brucei (TREU 927) PLA<sub>2</sub> - like sequence from partial mRNA, chromosome 9 and T. b gambiense (DAL 972 chromosome 9, PLA2 complete sequence) as shown (Table 1) signify that they are homologous. Also the translated protein sequence of the PLA<sub>2</sub>-like gene from T. b. brucei (Fig. 2) revealed identities and similarities in the conserved domains region with members of the Platelet Activity Factor Acetyl Hydrolase (PAF-AH) super family. These findings agree with previous reports [9]. This further substantiated by the report that PAF-AH is a subfamily of the PLA<sub>2</sub> super family responsible for inactivation of platelet-activating factor through the cleavage of an acetyl group [22,23]. The conserved domains among the protein sequences were presented (Fig. 3) revealing that large portions of the sequence were conserved. The sequential order of the conserved domains in the protein sequence was also conserved as revealed by the CDART analysis. The sequence of PLA2 like protein from T. b. brucei was similar in architecture (Fig. 4) to the sequences of PLA<sub>2</sub> from T. cruzi, M. anisop, M. acridu and PAF-AH in the Genebank DB. This implies that the sequences are similar in architecture and not just ordinarv sequence similarity. Since the conserved Domain Database brings together several collections of multiple sequence

alignment with conserved domains [22] and the CDART performs similarity searches based on the sequential order of the conserved domains, get them grouped and scored by architecture [21], then the PLA<sub>2</sub> like gene (JN603736) used in this study was a PLA<sub>2</sub> gene of the T. b. brucei. Other important features conserved in the studied sequence were the lipase motif (GHSHG) and the motif (FSHGL) peculiar to PLA<sub>2</sub>s. The sequences of PLA<sub>2</sub> from *T. brucei* (TREU 927), T. b. gambiense, T. cruzi, Leishmania major and characterized PAF-AH shared consensus sequences along with the conserved motifs with the query protein as produced by the NCBI BLASTX sequence alignment. This further elaborated the sequence similarity of the PLA<sub>2</sub> like protein from T. b. brucei with the PLA<sub>2</sub> super family as a justification that it was a PLA<sub>2</sub> gene that was amplified and studied. This is another case of homology which had been reported to be common among the members of the PLA<sub>2</sub> super family [1,24]. These conserved domains and motifs are similar to those reported in a study using T. brucei (strain EATRO 427 clone MITat 1.2) [25]. Also, the use of Trans-sialidase-like gene from the bloodstream form of Trypanosoma evansi that conserved most of the active siteresidues and motifs found in Trypanosomal sialidases and trans-sialidases has been reported as a basis for homology [26].

1       2497689       31       (11)       CARENTTIACREGENERG       CTT. (11)       NTTOA       A       EDSF       LULYTIS (11).D       72         71       2497686       48       (11).TSFGHTTIFERGEYFYCC       CTD. (11).HEGYT       N       ESSF       VELYTPS       Q       87         91       2497686       48       (11).GYSCKTOLPACCELVC       CCD. (11).HEGYT       N       QSSF       LELYTPS       Q       88         91       74638851       3. (11).GYSSKGULPACCELVC. (13).CYY.(11).TEHK       L       RTWK       VPLTYPC.(11,K.(11).40       69         91       74638859       4. (11).ISSPOLTENERGEYFYC       CED.(11).HEGKI.(11).CYV.CSVTEASMANDALLEY       ELITTRE       <	00101737		75	LII LUSPLI	DI. LKP TOOP	VSVC	1.3261	MNICCD	S OSTE	III VAURYP	<b>1 1 1 1</b>	11 1	
1       24397685       47       (11)       ACCENTRATIGNEESPEC       CTD. (11)       HECKY       WELVYEA       100       577         1       24397685       48       (11)       CSTCHTTIFKENGEYSEC       CTD. (11)       HECKY       N       SSTF       UPLYYEA       110.       GSST       PELYYPES       0       88         1       6417638451       3       (11)       CSTCHTIFKENGEYSEC       CTD. (11)       HECKY       N       VERYPPE (31).       K. (11)	ani 2497		21		TRACKCR	WATC	CTD (1)	MECTA	A RCCC	IDIVVI	2 (1) D		5
i 2497686       48       [11].TSTGHTTINKGNOTVSUC       CTD. [11].HSCYT       N       0587       LDLYTPS       0       588         i 6464781       1. [11].CSTGNUTCEPHUCC       CCD. [11].HECGN       L       0CSF       PDLYTPC. [11, R. [11, 63         gi 74638851       3. [11].GSTSKUDPATCOPLUCC, [13].CSY. [11].HTERK       L       TVK       VPITYPC. [11, R. [11, 64         gi 24336859       4. [11].ISSPQITTRUPCOPRUC       CED. [11].HTERK       L       TVK       VPITYPT. [21, 0, [11]. 65         guery       118       PERSCLP       YUPFG. [5].CVANYANV       PFFIRD. [11].SFURISSERANVPAALLNOY       FR       17         gi 2437686       39       DDTNET. [11].WIDDRE. [3].CGSTRINU [11].BALCERL [11].OFVCCUTTAKESIAAFDC       FE       12         gi 2437686       39       DDTNET. [11].WIDDRE. [3].CLASYLOY [11].BALCERL [11].OFVCCUTTAKESIAAFDC       FE       12         gi 2437686       39       DDTNET. [11].WIDPRE. [3].CLASYLOY [11].KERCCLL [11].MLLAWYSENDETC       FE       14         gi 2437686       49       DDTNET. [11].WIDPRE. [3].CLASYLOY [11].KERCCLL [11].MACCCUTLWENDESIGAFTK       [11].KUNCCUTAKENDESIGAFT       [11].         gi 2437686       49       DDTNET. [11].WIDPRE. [3].CLASYLOY [11].KERCCLL [11].MLLAWYSENGTERT       [11].       [11].GTSSL       [11].GTSSL       [11].GTSSL       [11].GTSSL       [1	gi 2497	688	47	[1] MCSCHS	SK T DK (M) (SS	YPUG	CTD (1)	MEGYC	N ROUT	UDLVYD	· · · · · ·		5
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g1       74838851       3       111. LEPSKLUPPKL COLVAC. [15]. L1. 11]. HERK L       L       L10. K       VML F1PL. [3]. K. [1]. 60         g1       741436253       4       (11). ISEPQUITEQUECONC       CED. (1). HIERK L       L10. L       SOLF       HELVEPT. [2]. [3]. (K. [1]. 60         g1       74136253       4       (11). ISEPQUITEQUECONC       CED. (11). HIERK L       SOLF       HELVEPT. [2]. [3]. (K. [1]. 60         g1       2437683       80 DQGLDT. [1]. WIENKE. [3]. CLSFPIGT. [1]. STUCNIL       HLUYCSLTTPASMISPLATC       EK       14         g1       2437688       80 DQGLDT. [1]. WIENKE. [3]. CLSFPIGT. [1]. STUCNIL       HLUYCSLTTPASMISPLATC       EK       14         g1       2437686       89 DNDFPDA. [1]. WIENKE. [3]. CLSFPIGT. [1]. STUCNIL       HLUYCSLTTPASMISPLATC       EK       14         g1       2437686       101       WIENTPE. [3]. CLSFPIGT. [1]. STUCNIL       HLUYCSLTTPASMISPLATC       EK       14         g1       2437686       101       WIENTPE. [3]. CLSFPIGT. [1]. MERCOLL. [1]. NEWLEAL, [1]. ASCLTMLAPYCREWENDERMETK       EK       11. [1].         g1       74638851       61       WERTED. [1]. WIENGL. [2]. CCSTPLADLAARGALVICLEMENDERSAS. [3]. F. [1]. EDS. [2]. ESE       V 228         g1       2437668       143       YP       LUVFSHCLCAFFTTYSAICTENASGATACHERDERSAS. [3]. F	g1 6647	0051	÷			ALCO CA		. FIRCE UN	L QUEST				ĩ
gi 249358599       4.11.1.ISSPQLITENERPORT       CHD.111.HERM.(11.C)       Solar       HBDJPP1.(21.8.11).48         guery       118       PERGUL TO NUCCEPTONC       CHD.11.HERM.(11.C)       Solar       HBDJPP1.(21.8.11).48         gi 2497689       73       DIDTERF.(11).WIDEKE.(3).CLSDELW)       HDDJPP1.(21.8.11).48       HDDJPP1.(21.8.11).48         gi 2497688       88       DOCRLDT.(11.WIDEKE.(3).CLSDELW)       HDLTP1.(11.WIDEWE.(3).CLSDELW)       HDLTP1.(21.8.11).48         gi 2497686       99       DDDTEFET.(11.WIDEKE.(3).CLSDELW)       HDLTP1.(21.8.11).48       HERTPP.(21.8.11).48         gi 2497686       94       DIDTERFET.(11.WIDEWE.(3).CLSDELWOV.(11.BLCCCLL.(1).MLAVCEWLDVEWEDEPHTK       DS.(11.10)         gi 2497686       144       FETTEROP.(11.WIDEWE.(3).CLSPENC.(11.MEDCCLL.(1).MLAVCEWLDVEWEDEPHTK       DS.(11.10)         gi 2497689       129       TOPSELIT.(11.WIDEPE.(3).CLEWIDCCL.(11.SUTICKEWIDCIDNAADLETK.(11.DK)       DO         gi 2497689       129       YP       LVVFSHCLACYHLFYSCPALDLAARGAIVICLCHCDNSAS       F.(11.RCKA.(21.EFE.(7).E 192         gi 2497688       144       YP       LIVFSHCLACFFTIYSCPALDLAARGAIVICLCHCDNSAS       F.(11.ROV.(21.KWIDER.(21.EKVK       N 199         gi 2497686       144       YP       LIVFSHCLACFFTIYSCFALDLAARGAIVICLCHCDNSAS       F.(11.ROV.(21.KWIDER.(21.EKVK       N 199	g1 7483	88851	3	. [1]. GFSSRI	QLPATC GP	LPVG. [1	3]. CEI.[1]	. ILERK	L RIVE	VRIFIP	1. 131.K. I	11. 60	2
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guery         110         PERGULP         YUP FCD. (5). SUVAYANU         PEFFIED. (1). SPUFIEASENAUVAALLENCY         EE         17           git 2497689         30         DIDTEFFICI.(1). WIPNEE. (3). CLSDENUX.(1). DALCENI.(1). UNIVESTICDAKSHAAPENDE         EK         14           git 2497688         80         DORDFPOL.(1). WIPNEE. (3). CLSDENUX.(1). SPLOKLL         HLVYCSUTTPASUMSPLUTC         EK         14           git 2497688         90         DNDFPDA.(1). WIPNEE. (3). CLSTEIGA.(1). SPLOKLL         KLVYCSUTVPASUMSPLUTC         EK         14           git 2497684         90         DNDFPDA.(1). WIPNEE. (3). CLSTEIGA.(1). SPLOKLE KLVYCSUTVPASUMSPERTK         DS. (1). 10           git 2497689         19         TOPSSUP.(1). WIPPPE.(3). CLSTEIGA.(1). SUVICKNUDCIDNAOLSTK.(1).DK         100           git 2497689         129         YP         LVYPENCLACYTLFYSCFALDLAARGALVICLCHCDNSAS         F. (1). RCKA.(2). EFE. (7).E         192           git 2497688         143         YP         LVYPENCLACENTTYSCFALDLAARGALVICLCHCDNSAS         F. (1). RCKA.(2). EFE. (7).E         192           git 2497688         143         YP         LVYPENCLACENTTYSCFALDLAARGALVICLCHCDNSAS         F. (1). RCKA.(2). EFE. (7).E         192           git 2497688         144         YP         LVYPENCLACENTTYSCFALDLAARGALVATENDERSAS.(3).F. (1). RCW.(2). EFE. (7).E         192	gn 2143	1822	4	.(1).1880QV	ALTEQUE CQ	FUVC	CKD . [1]	. MIDGT. [1].	لاسلخانيا . (2) . سل	MDL YFD.	7. (≥). <b>Q</b> . (	11. 50	-
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g1       745.38851       61       VEPRIDE.[1]. ULPHE       CLPEARC, [1]. PROLUCE, [1]. SECLIPLAL /VIRUELER, P. [2]. CK       11         g1       745.38851       61       VEPRIDE.[1]. ULPHE       CLPEARC, [1]. PROLUCE, [1]. SECLIPLAL /VIRUELER, P. [2]. CK       11         g1       745.38851       61       ADISSYF. [1]. ULPHE       COCSTICAL (1]. PROLUCE, [1]. SECLIPLAL /VIRUELER, P. [1]. P. [2]. CK       10         g1       745.38851       61       ADISSYF. [1]. ULPHE       CSTACL, [1]. PROLUCE, [1]. SECLIPLAL /VIRUELER, [1]. PROLUCE, [1]. PROLUCE, [2]. SEC       V       228         g1       2497668       144       YP       LIVEFSHCLCAFFT TYSALCT REASOFT. // ATOFHDESAS. [3]. F. [1]. PROV. [2]. ESC       N 200         g1       2497686       144       YP       LIVEFSHCLCAFFT TYSALCT REASOFT. // ATOFHDESAS. [3]. F. [1]. PROV. [2]. ESC       N 200         g1       2497686       104       YP       LITEFSHCLCAFFT TYSALCT REASOFT. // ATOFHDESAS. [3]. F. [1]. PROV. [2]. ESC. [7]. E       N 200         g1       74638651       10       YP       LITEFSHCLCOSTITYTYTCTSLASHCFTVAAREHDESAC. [3]. F. [1]. PROV. [2]. ESC. [7]. E       N 200         g1       2497669       193       (5]. FROULEOSTITYTTCTSLASHCFTV	g1 6647	0.000			. WIPPIES.	(3).000	BILGB. (L).		. MEAU COULD	POSMAGPPRIK	100 000		2
gi 214358699       49 TGPSSLP.[1]. WIPPPE.[3]. GVGETLCH.[1]. PHEDLI.[1]. SLVTGRKWDCINAQLSTK.[1].DK       10         gi 21431822       51 ADTESTY.[1]. WIPPPE.[3]. GUGETLGA.[1]. SQUAWI.[1]. SLVTGRKWDCINAQLSTK.[1].DK       10         guery       174 VP.[1]. UVPENCLACYEL PYSCFALDLAAGCAUVICLEHCDNSAS       F.[1].EDSS.[2].ESE       V 228         gi 22497689       143       PT       LUVPENCLACYEL PYSCFALDLAAGCAUVICLEHCDNSAS       F.[1].EDSS.[2].ESE       V 228         gi 22497689       143       PT       LUVPENCLACHT ICIENKOGGELVAAATHENESAS.[3].F.[1].EGCM.[2].KVE       N 199         gi 22497685       144       P       LUVPENCLAFFETTYSAIGTILAENGGELVAAATHENDESAS.[3].F.[1].MCGM.[2].KVE       N 199         gi 2497685       101       P       LITFENCLAFFETTYSAIGTILAENGGELVAAATHENDESAS.[3].F.[1].MCGM.[2].KVE       N 200         gi 2497685       101       P       LITFENCLAFFETTYSAIGTILAENGGELVAAATHENDESAS.[3].V.[1].DOPP.[2].ESC       N 200         gi 2497689       106       VIVFFENCLOCSNITYSTSICTILAENGFLVAATHENDESAS.[3].V.[1].MDAP.[2].LVE       K 162         gi 2497689       106       VIVFFENCLOCSNITYSTCTSLASHGYVVAATHENDESAS.[3].V.[1].TENT.[2].LVE       K 162         gi 2497689       103       (5).FRKU.[2].REAGVAQUESUKGTLORLTERD       FWTIL       GY       INSDID       KFLSKPL 282         gi 2497688       103       (5).FRKU.[8].RRGQVQQAPECTHALSHL	gi 7463	38851 (	61	VEPRIDE. [1]	. WLPFHE	GIP	EVARG. [1].	RUGLLRA. [1]	ASGLTNLAL	PVYRGELFHPP	[2].GR	11	÷
git 21431822       SI AD DESTP. [1]. SLEPP. [3]. GLGSTLG. [1]. SQUARD I. [1]. STVORMARD I BADMENT. [1]. DK       10         git 2497669       L29       YP       LIVYESHCL GAPET TYSAL CI EMA SOGELVAANEHEDESAS. [3]. F. [1]. ROCK. [2]. EPE. [7]. E JS2       21         git 2497669       L29       YP       LIVYESHCL GAPET TYSAL CI EMA SOGELVAANEHEDESAS. [3]. F. [1]. ROCK. [2]. EPE. [7]. E JS2       21         git 2497668       L44       YP       LIVFENCL GAPET TYSAL CI EMA SOGELVAANEHEDESAS. [3]. F. [1]. ROCK. [2]. KWE       N 199         git 2497668       L44       YP       LIVFENCL GAPET TYSAL CI EMA SOGELVAANEHEDESAS. [3]. F. [1]. ROCY. [2]. KWE       N 199         git 6647691       II       PLIFFENCL CAFFET TYSAL CI EMA SOGELVAANEHEDESAS. [3]. F. [1]. ROCY. [2]. KWE       N 199         git 2497666       L44       YP       LIIFFENCL CAFFET TYSAL CI EMA SOGELVAANEHEDESAS. [3]. F. [1]. ROCY. [2]. KWE       N 199         git 2497666       L0       VIVESHCL COSSIT FYSTYCT SLASHCOVVAANEHEDESAL. [3]. F. [1]. ROCA       EEM. [7]. 6       EEM. [7]. 6         git 2497669       L9       LS       LVESKL COSSIT FYSTYCT SLASHCOVVAANEHEDESAC. [3]. G. [1]. TERM. [2]. LVE       Q 164         git 2497669       L9       LS       LENCOVODALECSAL SANEOVVAANEHEDESAC. [3]. G. [1]. ROLAD LVESKL [2]. LVE       Q 164         git 2497669       L9       LS       LVESKL [2]. REAVENDESAC. [3]. G. [1]. R	gi 7495	8699 .	49	TGPSSLP. [1	. WINBER	[3].0/6	RAPPH [T] .	PHUMDLI.[1]	. SLVIGDERV	DCIDNAQLSTR.	[1] DR	10	2
query       174       VP. 11). IVVFSHCLACYELFYSCFALDLAARGATUTCLCHCONSAS       F. (1). EDSS. (2). ESE       V 228         gi 2497699       129       VP       LUVFSHCLACYELFYSCFALDLAARGATUTCLCHCONSAS       F. (1). ROCA. (2). EPE. (7). E 192         gi 2497689       143       VP       LUVFSHCLAGFETTYSAICIELASGGELVAAMEHEDESAS. (3). F. (1). ROCA. (2). EPE. (7). E 192         gi 2497685       144       VP       LUTFSHCLAGFETTYSAICIELASGGELVAAMEHEDESAS. (3). F. (1). ROCA. (2). ESC       N 200         gi 2647691       101       VP       LITFSHCLAGFETTYSAICIELASGETVAAMEHEDESAS. (3). F. (1). ROCA. (2). ESC       N 200         gi 2647691       101       VP       LITFSHCLAGFETTYSAICIELASGETVAAMEHEDESAS. (3). F. (1). ROCA. (2). ESC       N 200         gi 219       (5). CMEV. (2). REAGVAQVSEVCTLORLTERD       FWTIL       CT       INSDID       KFLSKPL 282         gi 2497669       169       VIVFSHCLOCOSTITYSTYCTSLASHCOVVAAMEHEDESAC. (3). G. (1). TERM. (2). LVE       K 162         gi 2497669       169       (5). LFKV. (8). REGOVQQAPECTHALBLIENTS       SGEEV. (1). NV       LNSPD. (1). NHLAD-SV 255         gi 2497668       201       (5). STRGL. (9). REGOVQAPECTHALBLIENTS       SGEEV. (1). NV       LDSSFT. (1). ROLEAL 260         gi 2497668       201       (5). REGOVGAPECSALSALIDIE       EGEVV. (1). NV       LDSSFT. (1). ROLEAL 260         g	gi 2143	1955	51	AD ISSYP. (1)	.wrbebb	[3].GLG	SYLGQ. [1].	SORMINVI. [1]	. STVVGERRE	DCIENAQMSTR.	[1] DR	10	2
3437669       129       YP       LVYPENGLGAPFTITYSAICTENASOGFLVAATEHEDESAS. [3]. F. [1]. EGGA. [2]. EPE. [7]. E 192         91       2497688       143       YP       LIVFSHCLGAPFTITYSAICTENASOGFLVAATEHEDESAS. [3]. F. [1]. EGGA. [2]. EVE [7]. E 192         91       2497688       144       YP       LIVFSHCLGAPFTITYSAICTENASOGFLVAATEHEDESAS. [3]. F. [1]. EGGA. [2]. EVE [7]. E 192         91       2497686       144       YP       LIVFSHCLGAPFTITYSAICTENASOGFLVAATEHEDESAS. [3]. F. [1]. F0007 [2]. EVE [7]. E 178         91       2497686       144       YP       LIIFSHCLGAPFTITYSAICTENASOGFLVAATEHEDESAA. [3]. F. [1]. F0047 [2]. EEN [7]. 0 [42         91       2497686       144       YP       LIIFSHCLGAPFTITYSAICTENASOGFLVAATEHEDESAA. [3]. F. [1]. F0047 [2]. EEN [7]. 0 [42         91       2497685       100       VIVFSHCLGSSTFTYSTYCTSLASOGTVVAATEHEDESAC. [3]. F. [1]. F0047 [2]. EEN [7]. 0 [46         91       2497689       193       [5]. CEWF [2]. FRAOVAOUSEVECTOLOLITED       FWT L       CY         91       2497688       201       [5]. VEVC. [6]. FROUNDERGESANLANTERTOPISAC. [3]. N. [1]. NUEARD [1]. KDLESS       260         91       2497668       201       [5]. VEVC. [6]. FROUNDERGESCANLSHLINTE       SEEW. [1]. NV       LNEARD [1]. KDLESS       260         91       2497668       201       [5]. VEVC. [6]. FROUNDERGESCANLSHLESD       ECEWV	crue rsz	1.	74	VP. (11. TVV)	SHGLAGYH	LEYSCEA	LDLAARGATU	TCLGHCDNSAS	F. [1]	BDSS [2] 8	SR V	228	
1       2497688       143       TP       LVFSHELGAFFITYSAIGTELASNETVATURHEDESAS.       31. F. [1]       EDOW, [2]. KVK       N 199         1       2497686       144       TP       LITFSHELGAFFITYSAIGTELASNEGFIVAAUEHHDESAA.       31. F. [1]       EDOW, [2]. KVK       N 199         1       2497686       144       TP       LITFSHELGAFFITYSAIGTELASPEGFVAAUEHHDESAA.       31. Y. [1]       DOAP. [2]. ESG       N 200         1       2647691       101       YP       LITFSHELGAFFITYSAIGTELASPEGFVAAUEHHDESAA.       [3]. Y. [1]       DOAP. [2]. ESG       N 200         1       244978691       10       UV       VIVFSHELGCSNTFYSTYCTSLASHGTVVAAUEHHDESAC.       [3]. K. [1]. VERM. [2]. LVF       K 162         1       21497669       193       [5]. CWEV. [2]. REAQVAQUEVEVCTLQRLTEND       FWTTL       GY       INSDID       KFLSKPL 282         1       2497668       200       [5]. KWGU. [6]. REGQVQQAPECTRALNETIKTS       SGERV. [1]. NV       LNSSPD. [1]. NHLM-SV 255         1       2497686       201       [5]. JVKGU. [6]. REGQVQAPECTRALNETIKT       SGERV. [1]. NV       LDSSPD. [1]. NHLM-SV 255         1       2497686       201       [5]. JVKGU. [6]. REGQUEQAPECTRALNETIKOV       GOTV. [1]. NV       LDSAFD. [1]. NULDESAFD. [1]. NULDESAFD. [1]. NULDESAFD. [1]. NULDSAFD. [1]. NULDSAFD. [1]. NULDSAFD. [1]. NULD		200 1	20	YB 1.1.171	POLICI, CA PD	TTYGATC	TEMAGOGELU	ANDIDDDCAG	121 8 (1)	WWWA (2) PI	9 (7) P	192	
1       2497686       144       T       LIIFSHELGAFFEITYSALGILASHGEFUAAVEHEDESAA.       [3]. Y. [1]. (D.AP. [2]. ESC.       N 200         1       24976879       101       Y       LIIFSHELGAFFEITYSALGILASHGEFUAAVEHEDESAA.       [3]. Y. [1]. (D.AP. [2]. ESC.       N 200         1       74638851       116       LP       VFIFSHCLUCSINUTYSLCTILASHGEFUAAVEHEDESAA.       [3]. Y. [1]. (D.AP. [2]. ESC.       [7]. (2]. ESC.       N 200         1       74638851       116       LP       VFIFSHCLUCSINUTYSLCTIASHGEFUAAVEHEDESAC.       [3]. Y. [1]. (D.AP. [2]. ERF. [7]. E 178         1       74638851       106       VFIFSHCLUCSINTYSTYCTSLASHGEVUAAVEHEDESAC.       [3]. X. [1]. VERMI. [2]. LVE       K 162         1       2497689       108       [5]. CWEV. [2]. REAQUAORVSEVECTLORLTEND       FWT TL       GY       INSDID       KFLSKL       282         1       2497689       108       [5]. UNC. [6]. REGOUGARETALELIENTS       SCR W [1]. NV       LENDID.       KFLSKL       282         1       2497689       103       [5]. UNC. [6]. REGOUGARETALELIENTS       SCR W [1]. NV       LENDID.       KFLSKL       283         1       2497689       103       [5]. UNC. [6]. REGOUGARETALELIENTS       SCR W [1]. NV       LENDID.       KK 162       SCR W [1]. NV       LENDID.       SCR W	gi 2497	2000 1	42	YD LTU	COLOL CARD	TTYCATC	TCLASNORTH	ATIVIIDDDCAC	[2] V [1]	ROOK [2] M	792 13	199	
94       664/7651       101       PD       LITPENCL CAPPTINGLAP CONLIASE COPULATION DEPROPERATION AL (1), F. (1), COAP       101, P. (1), COAP         91       746/38851       110       LP       VITPENCL CAPPTINGLAP CONLIASE COPULATION DEPROPERATION AL (1), F. (1), COAP       EEM. (7), O       1622         91       746/38851       110       LP       VITPENCL COSENTY SELECTIASE COPULATION DEPROPERATION AL (1), VERM. (2), LWE       K       162         91       21431622       100       WP       VIVPENCL COSENTY SELECTIASE COPULATION DEPROPERATION AL (1), VERM. (2), LWE       K       162         91       21431622       100       WP       VIVPENCL COSENTY SELECTIASE COPULATION DEPROPERATION AL (1), VERM. (2), LWE       K       162         91       2497668       100       (5), LWE. (5), REACON QUE ADECEMALENTIATE       SOE SV. (1), NV       LNSPD.       KFLSKPL 282         91       2497668       201       (5), LWE. (5), REACON QUE ADECEMALITIATE       SOE SV. (1), NV       LNSPD.       (1), RUEADIA 260         91       2497688       201       (5), REACON QUE ADECEMALICATE COPUT       GOT (1), NV       LDANTD. (1), QUECCEL 260       164 478       10), RUEADIA 260       10, RUEADIA 260         91       74638351       179       ISDF. [8], QUE RELEPROVE CLARICH LOBVT       GOT V. (1), NV       LDANTDP. (1), QU	gi 2497	202 1	44	VD LTT	CHCLCARD	STYSATC	TRIACHCETU	ANTENDERSAN	(2) V (1)	OD AD [2] R	76 N	200	
git         Constraint         Constraint <th>91 2457</th> <th>1000 1</th> <th>~</th> <th></th> <th>CINCLORE P</th> <th>511 3AL 0</th> <th>CLEAR STRUCT</th> <th></th> <th>. [0] [1]</th> <th></th> <th></th> <th>200</th> <th></th>	91 2457	1000 1	~		CINCLORE P	511 3AL 0	CLEAR STRUCT		. [0] [1]			200	
91       74538831       116       LF       091191120112816LF000000112816CF000000000000000000000000000000000000	gi 6647	0051 10	10	TP DIT	CINCLUS OF P	I DI SAF C	CTACTO	AVPENDD BOAR		DDDI (2) PI		102	
g1       243/28689       106       00       VLV/SSHLLGSSKIPTSTICTSLASHGTVVAAVEHUDSSLL.[3].K.[1].VERN.[2].LVE       Q 164         query       229       (5).CWEV.[2].REAGGSKIPTSTICTSLASHGTVVAAVEHUDSSLL.[3].K.[1].VERN.[2].LVE       Q 164         query       229       (5).CWEV.[2].REAGGSKIPTSTICTSLASHGTVVAAVEHUDSSLL.[3].K.[1].VERN.[2].LVE       Q 164         query       229       (5).CWEV.[2].REAGGSKIPTSTICTSLASHGTVVAAVEHUDSSLL.[3].K.[1].VERN.[2].LVE       Q 164         g1       2497688       200       (5).LIEW.[3].REAGVQQPAEGCIALILIKTS       SGERV.[1].NV       LNNEDDD       KFLSKPL 282         g1       2497688       200       (5).LIEW.[6].REGVQQPAEGCSALSAILDED       HDDWK.[1].NV       LONDDD       KFLSKPL 282         g1       2497688       200       (5).LIEW.[6].REGVQQPAECSALSAILDED       HDDWK.[1].NV       LONDDD       KFLSKPL 282         g1       2497688       200       (5).LIEW.[6].REGVQQPAECSALSAILSED       EDEV.[1].NV       LONDDD       (1).GUINCT       261         g1       2497688       163       (5).LUEW.[6].REGUPOED[CALCUTININ.[1].LIEDTD       (1).NL [5).VDSSFY.[1].GUINCT       (2).GUINCT       (1).GUINCT       (1).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT       (2).GUINCT <td< th=""><th>gi 7463</th><th>00051 1.</th><th>10</th><th>LP VELI</th><th>SHGLVGSP</th><th>NVISSLC</th><th>GILASIGIVV</th><th>LAHENRONSAL</th><th>- [3]. V</th><th>RUPL [2] - KI</th><th>18 - L / J - 15</th><th>1/0</th><th></th></td<>	gi 7463	00051 1.	10	LP VELI	SHGLVGSP	NVISSLC	GILASIGIVV	LAHENRONSAL	- [3]. V	RUPL [2] - KI	18 - L / J - 15	1/0	
G1         C1431822         C108         C1711         C1431822         C101         C1711         C1711 <t< th=""><th>gi 7493</th><th>00033 10</th><th>06</th><th>0P 0101</th><th>SHGLGGSR</th><th>IFISIIC</th><th>1 SLASPIGIVV</th><th>AAVEHRUSSAC</th><th>- [3] - [1]</th><th>VERN [2] . L</th><th>A R</th><th>162</th><th></th></t<>	gi 7493	00033 10	06	0P 0101	SHGLGGSR	IFISIIC	1 SLASPIGIVV	AAVEHRUSSAC	- [3] - [1]	VERN [2] . L	A R	162	
query         229         [5]. GWEV. [2]. REAGUADEVESURGTLORLTEND         FUTIL         CY         INSEDD         KFLSKPL         282           git         2497689         193         [5]. VFLML. [5]. PEROVQORABCITALDILIKITS         GGE EV. [1]. NN         INSEDD         KFLSKPL         282           git         2497688         200         [5]. LPEW. [8]. PEROVQORABCITALDILIKITS         GGE EV. [1]. NN         UNSEDD. [1]. NHLEDSV         256           git         2497686         201         [5]. VFLWU. [6]. PEROURDORECS CALSALLSID         DEEUV. [1]. NV         LOAMPL. [1]. OLIXOSL         261           git         24937685         163         [5]. VFLWU. [6]. PEROURDORECS CALSALLSID         DEEUV. [1]. NL         LDLNED. [1]. [0]. OLIXOSL         260           git         243376851         163         [5]. LVDR. [8]. REPOUNDERUSECIVLITIONU         ACQIV. [1]. NL         [5]. VDDSEYT. [4]. OSPECINZ. 24           git         21431822         163         [5]. LUBR. [8]. REPOUNDERUSECIVLITIONU         LOT VP. [1]. NL         [5]. VDDSEYT. [4]. OSPECINZ. 24           git         21431822         163         ESI REPUCORERARE CARAVITIREQIN         LOT VP. [1]. NL         [5]. VDDSEYT. [4]. OSPECINZ. 225           git         21431822         15         LIER K. [8]. REPUCORERARE CARAVITIREQIN         LOT VP. [1]. NL         [1]. CR	di 5143	31822 10	08	0P 1001	SHGLGGSP	TFISITC	TBLASHGTVV	AAVEHRDHSAC	. [3]. Q. [1]	.TERM. [2]. D	/B Q	194	
git         2497669         193         (5).VRKL. [9].REROYO QRAGECITALENLILITES         SCEEV. [1].NV         LNSPP. [1].NHLMP.SV 255           git         2497688         200         (5).LENV.[8].REROYO QRAGECITALENLILITES         SCEEV.[1].NV         LNSPP. [1].RULADAI 261           git         2497688         201         (5).LENV.[8].REROYO QRAGECITALENLIETE         BCDEX.[1].NV         LNSPP. [1].RULADAI 261           git         2497686         201         (5).REROYON         REROY CALSAL         ECEV.[1].NV         LDSRPP. [1].RULADAI 261           git         2497686         201         (5).REROYON         REROY CALSAL         ECEV.[1].NV         LDSRPP. [1].RULADI 260           git         6463851         [5].RERV.[5].REP QUERCES CALSAULESID         ECEV.[1].NV         LDSRPP. [1].RULADI 220           git         74638659         [6].SIDF.[8].RUEQUERDECLAULTIAUCEV         SCHW.[1].NV         LDSRPP. [1].RULADI 224           git         74638659         [6].SIDF.[8].RUEQUERDECLAUTIEDEL         CATUP.[1].NL.VV.[1].RULADI 244           git         74638659         [6].SIDERCECLAUTIECONDUC         CATUP.[1].RULADI 244           git         74378659         2.56         [3].E <examutieconduc< th="">         CATUP.[1].RULADI 244           git         7438659         2.56         [3].E<examutieconduc< th=""> <th< th=""><th>query</th><th>23</th><th>29</th><th>. [5]. GWEV.</th><th>[2]. REAQV</th><th>AQRVSEV</th><th>RGTLORLTER</th><th>D FWTTL</th><th>GY</th><th>INSDID</th><th>KF LSKPI</th><th>282</th><th></th></th<></examutieconduc<></examutieconduc<>	query	23	29	. [5]. GWEV.	[2]. REAQV	AQRVSEV	RGTLORLTER	D FWTTL	GY	INSDID	KF LSKPI	282	
12497688       200       [5]. LFRW. [8]. FREGVQ OPATECS PALSAILD TE       HCD FK. [1]. NV       LCSAFD. [1]. FOLKDAT 261         12497686       201       [5]. YFRW. [5]. FRFQLEQORGES CALSAILS TD       EEF W. [1]. NV       LCSAFD. [1]. FOLKDAT 261         15       YKVC. [6]. FRFQLEQORGES CALSAILS TD       EEF W. [1]. NV       LDLNFD. [1]. QOLKCSL 260         14       6647691       163       [5]. FRKW. [5]. RNP OWHORVSECL SWLKILGOEV       ACG TV. [1]. NIL [5]. VOSSFF. [4]. OSHECKL 244         15       YES 8851       179       ISDF. [8]. ONE FELL FRQQUEGI DIAL GUILINNIN. [1]. LCT FD. [1]. NIL [5]. VOSSFF. [4]. OSHECKL 244         15       Y4958699       163<. [5]. LVDR. [8]. RNE QUCKCRARE CARAVICI LEQLD       SCNVK. [1]. NV. [1]. ICHDAN. [1]. EF FKNKL 225         12       12437659       [5]. LIBK. [8]. RNE QUCKCRARE CARAVICI LEQLD       SCNVK. [1]. NV. [1]. ICHDAN. [1]. ACFENKL 225         12       12437659       256       [3]. R       IAVHICHSPCGATVL. [1]. ALEEN. [2]. PUCCVS. [6]. FDPWHVFU (NEHF. [116]). 447         12       2497658       256       [3]. R       IAVHICHSPCGATVL       GLASED. [2]. FNCCVA       IDPWHVFU (NEHF. [109]). 414         12       2497668       261       [3]. K       VALICHSPCGATVL       QLASED. [2]. FNCCVA       IDPWHFPVCREDVH. [109]. 414         12       46647691       263       [3]. K       VALIICHSPCGATVI	gi 2497	689 1:	93	. [5]. YRKL.	(9). RHKQV	QQRAQEC	TRALNLILKI	S SGEEV	. [1]. NV	LNSDFD . [1]	. NHLED SV	255	
1       2497666       201 . [5]. YKVC. [6]. FKRQLFORGERCSQALSWLLED       ECEFV. [1]. NV       LDLNFD. [1]. QQLKCSL 260         1       6467851       163 . [5]. YKVC. [6]. FKRQLFORGERCSQALSWLLED       ECEFV. [1]. NV       LDLNFD. [1]. QQLKCSL 260         1       74638851       179       ISDF. [8]. QME FLL FRQGE IG LAL (MINNIN. [1]. LCT FD. [1]. NL. [5]. VDSSFY. [4]. QSHCOLL 244         1       74538659       163 . [5]. LIKK. [8]. RNE QUERCHE CARAVIT LEDLD       SGWK. [1]. KV. [1]. IGNINA. [1]. EFF(NKL 225         1       74538659       163 . [5]. LIKK. [8]. RNE QUERCHE CARAVIT LEDLD       SGWK. [1]. KV. [1]. IGNINA. [1]. EFF(NKL 225         1       2437658       264 . [3]. K       VHIAGESFCGATVI ECHALMVLEQLN       LGT VF. [1]. KV. [1]. IGNINA. [1]. APFENKL 225         1       2437658       264 . [3]. K       IANTGESFCGATVI ECHALMVLEQLN       LGT VF. [1]. KV. [1]. IGNINA. [1]. AOFTENKL 225         1       2437658       264 . [3]. K       IANTGESFCGATVI ECHALMVLEQLN       LGT VF. [1]. KV. [1]. IGNINA. [1]. AOFTENKL 225         1       2437658       261 . [3]. K       VAIIGESFCGATVI ECHALMVLEQLN       LGT VF. [1]. KV. [1]. IGNINA. [1]. AOFTENKL 225         1       2437668       261 . [3]. K       VAIIGESFCGATVI ECHALMVLEQLN       LDWMERVFORDEVH. [109]. 414         1       2437668       261 . [3]. K       VAIIGESFCGATVI OT LSEN. [2]. FDCCIA       LDWMERVFORDEVH. [109].	gi 2497	688 20	00	. [5]. LRKV.	[8]. RKEQV	OGRAIEC	SPALSAILDI	E HGD PK	. [1]. NV	LGSAFD.[1]	ROLKDAT	261	
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Fig. 3 Conserved Domains in PLA<sub>2</sub>-like protein as aligned to members of PAF-AH super family. This was shown in red colour signifying high relatedness while domains that were weakly related were indicated in blue colour. Unaligned portions were left and indicated by numbers in brackets. The members of the PAF-AH super family used in the alignment were represented by their respective identification numbers

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# Fig. 4. Conserved domains architecture on PLA<sub>2</sub> like protein. Proteins with similar architectures include PLA<sub>2</sub> from *T.cruzi*, *Metarhizium anisop*, *Metarhizium acridu* and PAF-AH

The Alignment view (Fig. 1) revealed only substitutions in the PLA<sub>2</sub> like gene at positions 178, 810 and 970 that caused changes in the amino acids at the respective positions. These alterations did not appear in the active center (GHSFG) of the enzyme and so the activity of this enzyme may remain unaltered. This revelation agrees with conclusions that the nucleotide substitution can change the triplet sequence (codons) and hence can cause redundancy of the protein especially when it occurs on the catalytic domain [27]. However, the changes in such protein sequences may also occur at positions irrelevant for enzymatic activity [28]. These findings may provide ground for considering the enzyme a possible member of one of the classes in the superfamily.

#### 5. CONCLUSION

Conclusively, the presence of  $PLA_2$  and lipase motifs,  $PLA_2$  conserved domains and the high percentage identity and similarity of the  $PLA_2$  like sequence to some characterized  $PLA_2$ primary sequences indicate that it is a  $PLA_2$ homologue of the Platelet-activating factor acetylhydrolase superfamily. The unsuccessful expression of the recombinant  $PLA_2$  in BL-21 (DE3) competent *E. coli* cells as displayed on fractions obtained from the colonies resolved on SDS-PAGE suggests that alternative ways for the expression need to sought in order to biochemically characterize the gene product.

#### ETHICAL APPROVAL

Animal experiments were carried out in accordance with the instructions for the care and use provided by the university of Jos, Nigeria where the animal experiments were carried out. The experiments were examined and approved by the university of Jos ethics committee

#### ACKNOWLEDGEMENTS

We acknowledge the Tertiary Education Trust Fund (TETFUND), Abuja, Nigeria for supporting this work with grant. We appreciate the technical assistance of the staff of the Molecular Biology Laboratory, NVRI, Vom, Nigeria as well as the kindness of the staff of the Parasitology Department, NITR, Vom, Nigeria for supplying the parasites.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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