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Identification of a novel stress regulated FERM domain containing cytosolic protein having PTP activity in *Setaria cervi*, a bovine filarial parasite

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ABSTRACT

A 67 kDa cytosolic FERM domain containing protein having significant protein tyrosine phosphatases activity (PTPL) has been purified to homogeneity from *Setaria cervi*, a bovine filarial parasite. The MALDI-MS/MS analysis of the purified protein revealed 16 peptide peaks showing nearest match to *Brugia malayi* Moesin/ezrin/radixin homolog 1 protein and one peptide showing significant similarity with a region lying in the catalytic domain of human PTPD1. PTPL showed significant cross reactivity with the human PTP1B antibody and colocalize with actin in the coelomyrian cells of hypodermis in the parasite. PTPL was stress regulated as it showed marked decrease in the expression when exposed to Aspirin, an antifilarial drug and Phenylarsine Oxide, PTP inhibitor.

1. Introduction

The protein tyrosine phosphorylation is a dynamic process controlled by the balanced actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Though earlier mistaken as constitutively active housekeeping enzymes, PTPs have emerged as essential regulators of different cellular signalling processes like growth, differentiation and proliferation in coordination with PTKs [1]. The PTPs are classified into two broad groups, transmembrane and cytoplasmic. The transmembrane PTPs which respond to external stimuli, consist of a single transmembrane segment and two PTP domains and the cytoplasmic non-receptor PTPs, which exhibit a noncatalytic domain (apart from the catalytic PTP domain) that seems to manage its intracellular localization as well as substrate specificity [2].

One such group of cytoplasmic non-receptor PTPs possess a cytoskeletal associated domain termed as band 4.1 or FERM (band four-point-one, ezrin, radixin, moesin homology) at their N-terminal end. Human PTPs reported to belong to this family are PTP-BAS/FAP-1/PTPL1, PTPH1, PTP-MEG1, PTPD1/PTP-RL10, PTPD2/Pez. Recent reports have shown that the N-terminal domain of FAK and JAK tyrosine kinases also belongs to this band 4.1-domain family and is responsible for their association with PDGF – and EGF cytokine receptors, respectively. The main function of these proteins is to mediate linkage of the cytoskeletal protein, actin and transmembrane receptors like CD44, ICAM-1/2/3, ERM binding phosphoproteins 50 with the plasma membrane inner surface [3]. They are also known to associate the enzymatic activities of kinases and phosphatases to the membrane. Their varied role in cell–cell adhesion, cell–matrix interaction, cell morphology, metastasis, carcinogenesis as well as apoptosis via phosphatidylinositol 3-kinase/Akt pathway has been reported. These proteins are also known to control stress-response, growth and survival pathways of oncogenic importance [4].

The free living nematode, *Caenorhabditis elegans* also possess a PTPL1 protein known as PTP-FERM predominantly expressed in the neuron and peri-membrane space. The FERM domain of PTP-FERM was revealed to be sufficient and essential for ameliorating the protein in the subcellular region of the worm [3].

Regardless of the crucial significance of PTPL1/PTP-BAS, its characterization and importance in case of parasitic nematodes is still unknown. The present study describes the isolation, purification and role of a novel FERM domain containing protein with significant protein tyrosine phosphatases activity (PTPL) in *Setaria cervi*, a bovine filarial parasite.

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2. Material and methods

2.1. Parasite collection, crude extract preparation and purification

The procurement and preparation of adult female *S. cervi* extract was done as reported previously [5]. The adult female *S. cervi* crude soluble extract was subjected to purification using concanavalin A sepharose 4B affinity and gel filtration chromatography. The extract was loaded on affinity column and protein was eluted using 0.15 M NaCl and 1 M mannose. The active protein peaks were loaded further on the Sephadex G-200 column pre equilibrated with 50 mM 0.1 M Tris buffer, pH 7.0. The active protein fractions were checked for purity using SDS gel electrophoresis. The MALDI-MS/MS analysis of the purified protein band was performed at Interdisciplinary School of Life Sciences, Banaras Hindu University, Varanasi, India. ClustalW multiple alignment tool was used for the alignment studies.

2.2. Protein tyrosine phosphatase assay

Acid phosphatase and protein tyrosine phosphatase activity were measured according to the method described elsewhere [6,7] with slight modifications as described earlier [5]. The effect of different general and specific inhibitors of acid phosphatases (sodium orthovanadate, sodium fluoride, ammonium molybdate, sodium tartarate, phenylarsine oxide, okadaic acid, $ZnCl_2$) on purified PTP activity was determined as described elsewhere [5].

2.3. Western blotting

The purified protein was electrophoretically separated by a preparative 10% SDS-PAGE gel and electrotransferred onto PVDF membrane as described [8]. The monoclonal antibody (clone FG6-1G, Calbiochem, San Diego, CA) generated against the catalytic domain of a recombinant human placental PTPase 1B (1 μg/ml) as primary antibody and peroxidase conjugated rabbit anti-mouse IgG (1:5000) as secondary antibody were used as explained earlier [9]. ImageJ 7.0 software was used to quantify the western blot bands.

2.4. Tissue fixation and immunostaining

The adult female parasites were incubated at 37 °C, 5% CO2 for 4 h in Krebs-Ringer Bicarbonate (KRB) medium in the presence/absence of Aspirin and SK7, methylated chalcone. The parasites were then fixed in 4% paraformaldehyde for 20 min and dehydrated in graded series of ethanol. The parasite paraffin sections were cut at 6 μm using a Leica microtome. The sections were first rehydrated in different grades of ethanol and incubated in a blocking buffer (5% skimmed milk in phosphate buffer saline PBS) for 2 h at RT to avoid nonspecific binding. They were then incubated overnight at 4 °C with primary antibody (anti human PTP1B; 1 μg/ml) in phosphate buffer saline tween (PBST). After washing with PBST, they were incubated with secondary antibody (goat anti mouse IgG, FITC conjugated, 1:2000) in PBST for 2 h in dark to avoid photobleaching. The sections were then washed, incubated with phalloidin-TRITC (1:200) in PBS for 1 h at RT and mounted in DABCO. The images were taken using Zeiss LSM-510 Meta confocal microscope at the National confocal facility, Banaras Hindu University.

2.5. Statistical analysis

All experiments were performed in triplicate (n = 3). The statistical analysis was done using the GraphPad Prism 5.0 software. Statistical significance was determined by using two tailed Student's *t*-test. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Purification and characterization of PTPL in S. cervi

We have recently reported presence of significant amount of protein tyrosine phosphatase activity in the cytosolic extract of *S. cervi* from our laboratory [5]. The enzyme was found to possess highest affinity for O-P-L-tyrosine substrate as compared to O-P-L-serine, O-P-L-threonine and pNPP as a general substrate. The enzyme was glycosylated in nature as it showed affinity for the Concavalin affinity column [5]. While characterizing tyrosine phosphatases in *S. cervi*, we discovered a FERM domain containing protein in the cytosolic extract of *S. cervi* having significant tyrosine phosphatase catalytic activity and named it PTPL. PTPL was purified to homogeneity with a specific activity of 2124 U/mg, 90-fold purification, and yield of 23.4% (Table 1) using Concavalin A affinity and Sephadex G-200 columns. The apparent molecular mass of PTPL was determined to be 67 kDa when run on a 10% SDS–PAGE (Fig. 1A). The purified protein also displayed strong cross reactivity at 67 Kda using a monoclonal anti-human placental PTPase 1B antibody (with the epitope designed against the catalytic site of the protein) (Fig. 1A). The cytosolic crude extract of *S. cervi* also showed intense cross reactivity with this antibody at the same position (Lane Ctrl of Fig. 1B).

The optimum pH and temperature for PTPL was found to be 5.9 and 37 °C, respectively (data not shown). To analyse the substrate specificity of PTPL, we checked its affinity towards different substrates like pNPP, O-phospho − L-tyrosine, O-phospho − L-serine and O-phospho-L-threonine. PTPL showed highest activity with pNPP followed by O-phospho − L-tyrosine. It exhibited almost one third of activity with O-phospho-L-threonine as compared to O-phospho − L-tyrosine but no activity could be seen using O-phospho − L-serine (Table 2). To further characterize the PTPL, we performed inhibition studies using some class specific inhibitors of protein phosphatases. Sodium orthovanadate (SOV), Phenylarsine oxide (PAO) and $ZnCl_2$ were used as protein tyrosine phosphatases specific inhibitors. Okadaic acid as serine threonine phosphatase inhibitor and ammonium molybdate, sodium fluoride, sodium tartarate as general acid phosphatase inhibitor were also used for the inhibition studies. PTPL activity was completely inhibited by SOV at 100 μM. The PAO (1 nM) also significantly inhibited the enzyme activity upto 87.36%. Although the general acid phosphatase inhibitors, ammonium molybdate and sodium fluoride showed 93% and 98% inhibition, okadaic acid did not show any inhibition in activity (Table 3). The PTPL enzyme activity was enhanced by 47.43%. in the presence of DTT.

3.2. Bioinformatics analysis

Further the purified protein band was excised from the SDS-PAGE gel for peptide mass fingerprinting analysis. The maximum

Table 1
Purification Table of PTPL from adult female *Setaria cervi*.

	Total protein (mg)	Total activity (u)[a]	Specific activity (u/mg)[b]	% Yield	Fold purification
Adult female cytosolic extract	8.1	190.5	23.5	–	–
Con A sepharose 4B affinity column	0.35	123.83	354.36	65.00	15.08
Gel filtration column G-200	0.021	44.61	2124.29	23.4	90.39

[a] One unit of enzyme activity is defined as micromoles of p-nitrophenol produced ml^{-1} min^{-1} with pNPP.
[b] Specific activity is the units of enzyme activity per milligram of protein.

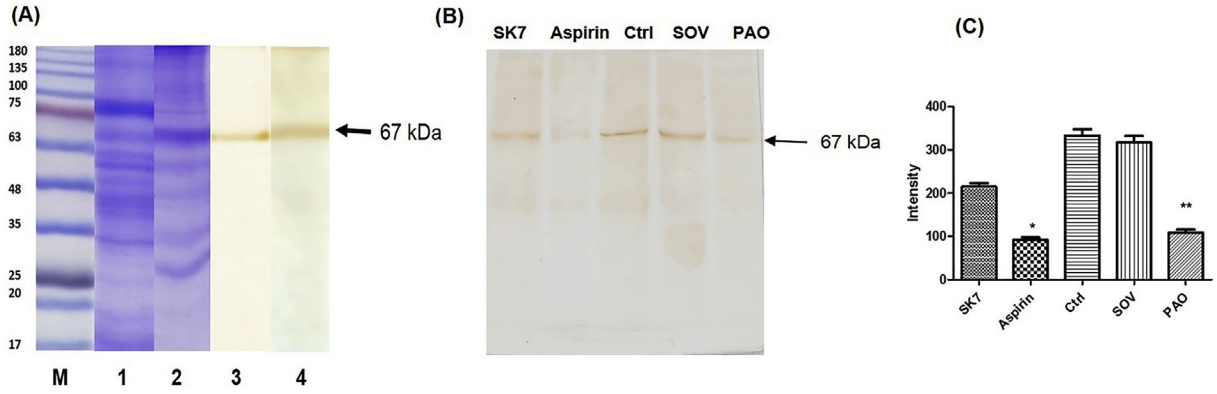

Fig. 1. SDS – PAGE and Western blot analysis of PTPL on 10% polyacrylamide gel. (A) Lane M: Prestained molecular weight marker. Lane 1: Cytosolic soluble extract. Lane 2: Con A affinity eluted fraction Lane 3: G-200 eluted fraction (silver stained). Lane 4: western blot of purified PTPL showing significant cross reactivity at 67 KDa using anti human PTP1B antibody. (B) Western blot analysis of the cytosolic soluble extract of S.cervi treated with antifilarial compounds; SK7 (a methylated chalcone) and Aspirin and Specific PTP inhibitors; Sodium orthovanadate (SOV) and Phenylarsine oxide (PAO). (C) Histogram showing the intensities of the band obtained on western blot by using ImageJ 7.0. software.

peptide peaks obtained were 50 and MASCOT database search engine was used to determine the homology of the peptides. A total of sixteen peptides were obtained showing closest match with the Moesin/ezrin/radixin homolog 1 protein from *Brugia malayi* (score 144) as shown in Fig. 2. Interestingly, the moesin/ezrin/radixin protein constitute the FERM domain in some of the protein tyrosine phosphatases (PTPL family) of *B. malayi*, *Loa loa*, *C. elegans*, vertebrates and insects. The different peptides obtained on MALDI-MS/MS analysis were further aligned with the *B. malayi* PTP containing the FERM domain. Only two predicted *B. malayi* protein sequences for FERM domain containing PTP were available in the uniprot database [A8PMP9_BRUMA] and NCBI database [*Bm*PTP; gi_170585338). The four out of eighteen peptides obtained in case of *S. cervi* showed similarity (35–50%) with the FERM domain of *B. malayi* PTP (Sequence with accession no. gi_170585338 was used in this case) (Fig. 3A). One of the peptides K.**QLFD**QVVK.T aligned with the amino acids **KLFS**, which form the region for binding of other peptides with the FERM domain of the protein (Fig. 3A). One peptide **(KGFLTWLKL)** having parent mass 864.5035 was found to be 78% similar to a region **(KGFLSYLEE)** lying within the active site of the human PTPD1 which also belongs to a PTPL family of proteins containing the FERM domain (Fig. 3B). The PTP catalytic domain which has been reported in the protein databases in case of *C. elegans* and *Homo sapiens* PTPL proteins, is not yet reported till date in case of the filarial parasites *B. malayi*, *L. loa* and *Wuchereria bancrofti*.

3.3. Expression and subcellular localization of PTPL in S. cervi

To examine the functional role of PTPL in *S. cervi*, the parasites were exposed to two antifilarial compounds, Aspirin and SK7 (a methyl chalcone) and two tyrosine phosphatase inhibitors, SOV and PAO. The western blotting analysis using the anti PTP1B antibody showed an altered expression of PTPL in all the cases. While Aspirin and PAO treated parasites showed significant reduction in expression, SOV and SK7 treated parasites did not display significant changes (Fig. 1B and C).

Further, the immunohistochemical studies using confocal microscopy were performed to study localization of PTPL inside the *S. cervi* parasites using the monoclonal anti human PTP1B antibody (for PTPL) and phalloidin – TRITC tagged antibody (for F – actin) in the fixed tissues. The F-actin was uniformly distributed with slightly high fluorescence in some of the microfilariae (Fig. 4A, panel A, D). The PTPL on the other hand was expressed predominantly in the hypodermis, coelomyrian muscle cells and microfilariae inside uterus (Fig. 4A, panel B, E). The expression of PTPL and actin was superimposing chiefly at the coelomyrian cells as compared to other regions of the tissue clearly visible by yellowish green fluorescence (Fig. 4A, panel C, D).

The expression pattern of PTPL and F-actin was also observed in case of fixed tissues of the parasites treated with antifilarial compounds, Aspirin and SK7. The aspirin treated parasites showed ruptured uterine walls and constricted hypodermal coelomyrian cells as compared to the control tissues (Fig. 4B, panel A–C). Although no alteration in the actin expression was observed as seen in Fig. 4B, panel A and D, a significant reduction in the intensity of green fluorescence was speculated in the coelomyrian cells and uterine walls (Fig. 4B, panel B, E). A merged image of both the fluorescence showed maximum overlay of red fluorescence at maximum places showing reduction in expression of PTPL (Fig. 4B,

Table 2
Substrate specificity of *Setaria cervi* PTPL.

Substrate	Activity[a] (Units/mL)
pNPP	24.06 ± 1.12
O-P-L-Tyrosine	21.47 ± 0.72
O-P-L-Threonine	6.14 ± 0.92
O-P-L-Serine	0.20 ± 0.10

[a] One unit of enzyme activity is defined as micromoles of p-nitrophenol produced millilitres per minute with pNPP and amount of free phosphate produced using O-P-L-tyrosine, O-P-L-threonine and O-P-L-serine as substrates. Values are Mean ± SEM of three different determinations.

Table 3
Effect of inhibitors and activators on *Setaria cervi* PTPL.

Inhibitor/activator	Final Conc.	Activity[a]	% inhibition/ activation
Control		25.15 ± 0.63	
Sodium orthovanadate	50 μM	8.81 ± 0.60***	−64.97
	100 μM	0	−100
Phenyl arsine oxide	1 nM	3.20 ± 0.64***	−87.36
Okadaic acid	10 μM	23.57 ± 0.87	−6.29
Zn2+	2.5 mM	10.05 ± 1.14***	−60.10
Ammonium molybdate	2 mM	1.78 ± 0.08***	−93.01
Sodium flouride	2 mM	0.63 ± 0.63***	−97.59
L (+) Tartarate	Upto 5 mM	20.82 ± 1.10	−17.23
DTT	2 mM	43.85 ± 1.21***	+74.43

*** signifies p value < 0.001. This values bearing *** are significant in comparison to the control.

[a] One unit of enzyme activity is defined as micromoles of p-nitrophenol produced millilitres per minute with pNPP.

(A)

gi|170593397 Moesin/ezrin/radixin homolog 1 [Brugia malayi]

MDAELEFAIQPSTTGKQLFDQVVKTVGLREIWYFGLQYTDTKGFLTWLKLNKKVTAQDVKKEQTLLFKFR
AKFYPEDVQEEIIQDITLRLFYLQVKDAVLSDEIYCPPETSVLLASFAMQAKYGDYNPESHKPGCLTSDR
LLPQRVIGQFKLSPEEWEKRIMVWWADHKNTNREQAMVEYLKIAQDLEMYGVNYFEIRNKKGTELFLGVD
ALGLNIYEKNDRLTPKVGFPWSEIRNISFNDKKFVIKPIDKKANDFVFYAPRLRINKRILALCMGNHELY
MRRRKPDTIEVQQMKQQAKEERMQRQLEQERLTKEMTAREAAEQKQKEYEERMEKMREEMERAQRELLHA
QDTIRRLEQQLAELQIAKEQLESKEDELRRLNEQLRSEREMSSEERERLEQEVRRREQQVAEMREQVDMK
TQETERLQREVEEARAVQERESSRQLNTFSVKEVDLDENASENGQVATELTARGDENVPQRELERITAAE
QNLSLKHKLDALTADLEAVKDKQQLTEYDLLHMENKRAGRDKYKTLRQIRGGNTKRRIDQYENM

(B)

Mass	Range	P sequence
725.3580	328-332	K.EYEER.M
760.3979	54-60	K.VTAQDVK.K
772.4469	381-386	R.LNEQLR.S
910.5498	90-96	R.LFYLQVK.D
914.4598	474-481	R.GDENVPQR.E
928.5430	380-386	R.RLNEQLR.S
959.5315	244-251	K.FVIKPIDK.K
976.5474	17-24	K.QLFDQVVK.T
980.5099	336-342	K.MREEMER.A
990.4807	407-414	R.EQQVAEMR.E
1068.5080	547-554	R.RIDQYENM
1185.5764	161-169	R.IMVWWADHK.N
1327.6590	252-262	K.KANDFVFYAPR.L
1472.7707	284-295	R.RKPDTIEVQQMK.Q
1539.8506	356-368	R.RLEQQLAELQIAK.E
1917.8908	513-527	K.QQLTEYDLLHMENKR.A

Fig. 2. *S. cervi* PTPL peptide sequences. (A) Based on MALDI-MS/MS, nearest match found to Moesin/ezrin/radixin homolog 1 [Brugia malayi]. (B) Matching peptides (bold face) along with their masses are given above.

panel C, F). The SK7 treated parasites, on the other hand showed yellowish green florescence almost all over the tissue suggesting only a slight alteration in the expression of PTPL and actin as compared to the control tissues (Fig. 4C, panel A–F).

4. Discussion

PTPL is a non-receptor cytosolic protein containing N terminal FERM domain and a C terminal PTP catalytic domain. While the C terminal contributes to the catalytic activity of the protein, the N terminal domain is essential to maintain the subcellular cytoskeletal organization by linking several transmembrane proteins to the plasma membrane [3]. The presence of the band 4.1/FERM domain in a group of phosphatases suggests the prominence of these domains in the functionality of the protein. Here, in our study we have isolated, purified and characterized a novel 67 Kda PTPL cytosolic protein in *S. cervi*, a bovine filarial parasite. The *S. cervi* is used as the model organism in this study because of its nocturnal periodicity and similar antigenic pattern as that of *B. malayi*, the human filarial parasite [9]. The two step column chromatography enabled us to purify the 67 kDa PTPL to homogeneity. The protein bound to a Concavalin affinity column suggesting its glycosylated nature. A recent study also reports the presence of N-glycosylation motif in HACD1protein in which PTPL protein is expressed during congenital myopathies [10]. The optimum pH and temperature for PTPL was determined as 5.9 and 37 °C respectively. Similar pH (5.0) and temperature (40 °C) value have been reported in case of PTP-MEG1 isolated from human bone marrow [11]. The molecular weight of PTPL in other species varies from 270 Kda to 30 Kda with mammalian PTPL1/PTP-BAS (270–275 Kda) as the largest known phosphatases among this group [4]. However, a PTP-MEG1 protein purified from human bone marrow exhibited a molecular mass of 32 Kda [11]. The substrate specific and inhibition studies further proved the relatedness of the purified protein to tyrosine phosphatase family of enzymes. The SOV and PAO significantly inhibited PTPL activity *in vitro* at 100 μM and 1 nM respectively. The orthovanadate might act as analogue of phosphate [12] and PAO might act by inhibiting the dithiol group present on the active site of the tyrosine phosphatase [13]. An increase in PTPL enzyme activity in the presence of DTT showed presence of thiol group on the active site of the protein. It is well known that tyrosine phosphatase possesses cysteine group in its active site which is required for its catalytic activity [1].

Further, the MALDI-MS/MS analysis of the purified protein revealed 50 peptide peaks out of which 16 peptides showed significant match to moesin/ezrin/radixin homolog 1 protein (ERM) of *B. malayi* and 1 peptide showed significant similarity to a region lying within the active site of the catalytic domain of human PTPD1 protein. It is well known that protein tyrosine phosphatases possessing an ERM domain belong to FERM (band four-point-one, ezrin, radixin, moesin homology) domain family known as PTPL1/PTP-BAS protein. This band 4.1/FERM domain consists of an actin binding, peptide binding and phosphatidyl inositol phosphate binding motif [14]. Four out of the above 16 peptides showed significant similarity to the putative *B. malayi* PTPL protein. Three peptides KEYEERM, KQQLTEYDLLHMENKRA and KKANDFVFYAPRL were found to be lying within the band 4.1 domain which plays regulatory and structural roles in the stabilization of plasma membrane domains. Another peptide KQLFDQVVKT coincides with a peptide binding motif "KLFS" which is responsible for binding to different kind of peptides present on the plasma membrane (www.ncbi/nih/nimgov.in). It has been reported that FERM domains when bound to PtsdIns(4,5)P2 at plasma membrane are phosphorylated by specific kinases leading to unfolding of the F-actin domain, thereby exposing it to link with the varied cytoskeletal and transmembrane proteins such as Rho GTPases, Wnt-βcatenin, cadherin and Akt kinases [14]. Thus, the presence of FERM domain containing proteins is often associated with the presence of different transmembrane and cytoskeletal proteins like actin. Only one report by Uchida et al. [3]

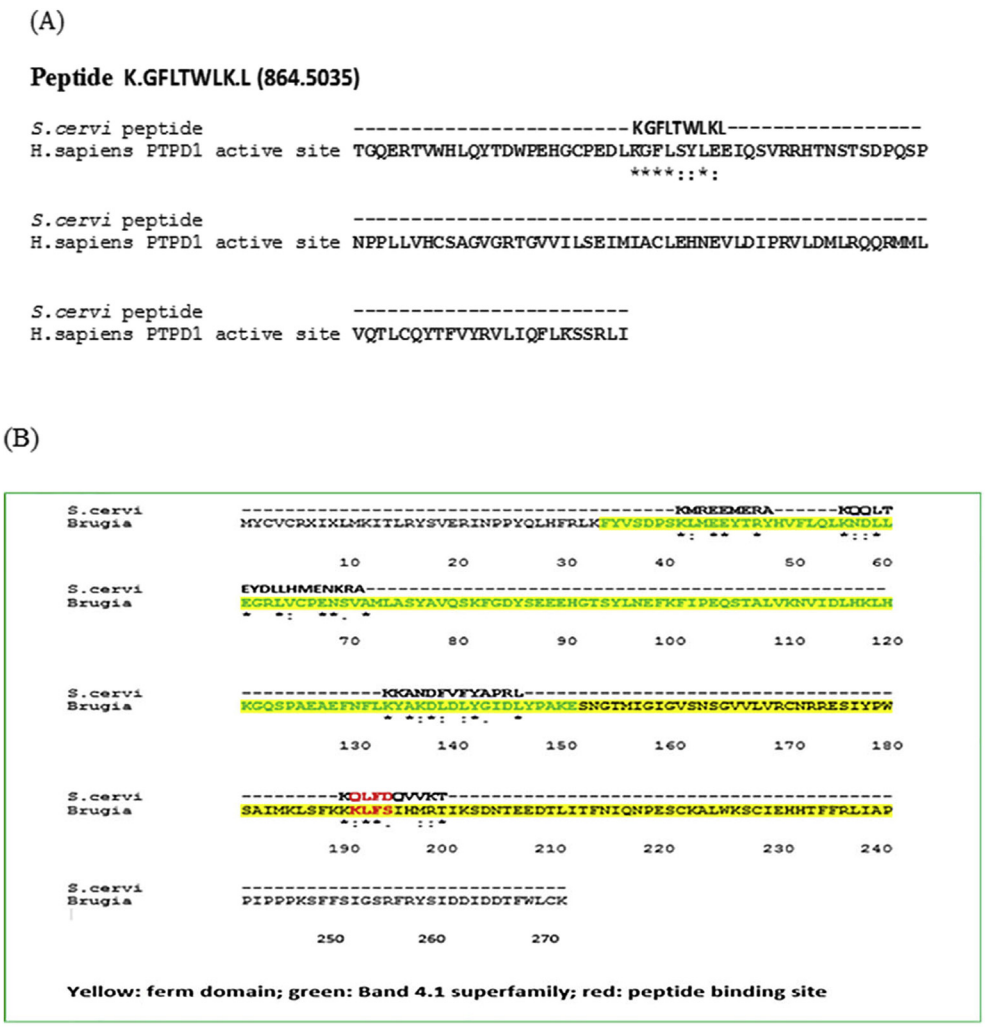

Fig. 3. Alignment analysis. (A) Four out of sixteen peptides obtained in case *S.cervi* showing homology and lying within the FERM domain of *Brugia malayi* PTP (Accession no. XP_001897441) [B] One Peptide (KGFLTWLKL) having parent mass 864.5035 showing 77.77% similarity to a region (KGFLSYLEE) lying within the active site of the human PTPD1 (Uniprot ID. Q16825).

established the importance of FERM domain in localizing the FERM PTP in the neurons and perimembrane region of the *C. elegans*, a free living nematode. Indeed, through immunohistochemical staining, we also found a co-localization of PTPL and actin in the fixed tissue sections of *S. cervi*. While a predominant green fluorescence of PTPL was found in the coelomyrian cells of hypodermis, uterine wall and microfilariae, we found a uniform red fluorescence of actin with slightly high expression in case of microfilariae in the tissue sections. There was simultaneous expression of both actin and PTPL (yellowish green fluorescence) at the coelomyrian cells depicting propensity of PTPL to localize with actin in the cytoskeletal structures of the parasite.

PTPL showed significant cross reactivity with the human PTP1B antibody (epitope lying within the conserved catalytic core of protein at a position 43-287 of the 435 aa. long). The explanation to this observation might lie in the fact that the catalytic core of all catalytically active class I human PTP is known to be similar to PTP1B itself [1]. The most striking feature of the PTP1B which explains this cross reactivity to the PTPL catalytic domain unlike LAR-PTP and PTPα is the occurrence of a second phosphotyrosine binding pocket in neighbour of the catalytic motif in both the proteins. This feature might aid PTPL1/PTP-BAS to dephosphorylate the plasma membrane localized insulin receptors which are bis-phosphorylated or other peptides with two adjacent phosphotyrosines with the same catalytic efficacy as that of PTP1B. It is also evident that insulin signalling pathway is one of the three survival mechanisms used by parasitic helminths to survive inside host [15]. This demonstrates importance of PTPL localization near the plasma membrane.

PTPL1/PTP-BAS is reported to possess both tumour activator as wells as suppressor properties in different cancer cells [4]. The reduction in expression of PTPL on western blot analysis of cytosolic soluble crude extract of *S. cervi* treated with Aspirin, SK7 and PAO reveals that this protein is stress regulated in this parasite. The expression of a protein tyrosine phosphatase, ACP-1 has also been reported to be reduced in presence of PAO earlier [16]. PAO has also been reported to be lethal for these parasites too [9]. PTPL1/PTP-BAS knockdown in the astrocytoma cells leads to increase in Fas-induced apoptotic cell death whereas its reduction in case of breast cancer cells induced cell survival pathways via increase of PI3-K activity and phosphorylation of AKT kinase [17]. These proteins are also known to modulate different cell-growth and stress pathways by acting as scaffold to varied molecules and substrates like FAS, Ephrin B1, IRS1, STAT4, HER2, etc [3].

The effect of two antifilarial compounds, Aspirin and SK7 was further seen on the expression of PTPL and F-actin in these parasites.

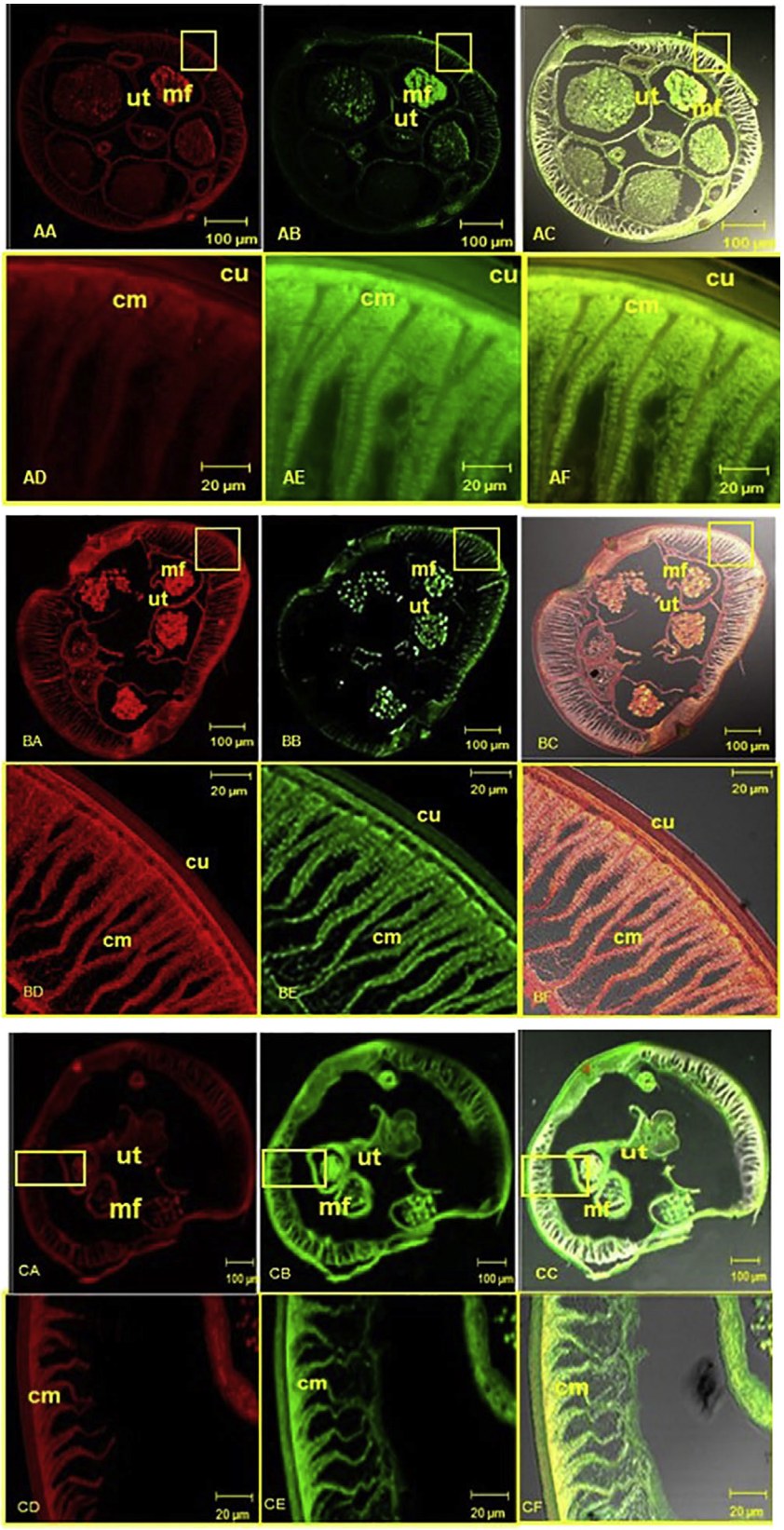

Fig. 4. Immunoconfocal study of the fixed tissue transverse sections of Setaria cervi. The fixed tissue sections were stained using monoclonal human PTP1B as primary, phalloidin-TRITC (for actin) and FITC-conjugated goat anti-mouse IgG as secondary antibody and visualized using confocal microscopy at 10× and 60× zoom. (A) Control parasite sections (A–C) at 10× magnification and (D–F) at 60× magnification. (B) Aspirin treated parasite sections (A–C) at 10× magnification and (D–F) at 60× magnification. (C) SK7 treated parasite sections (A–C) at 10× magnification and (D–F) at 60× magnification. Images A, D and B, E in all the panels shows F-actin (red) and PTP (green) fluorescence, respectively. Images C, F shows merged yellowish orange fluorescence where PTP and F-actin colocalize. Abbreviations used: coelomyarian muscle cell of hypodermis (cm), cuticle (cu), microfilaria (mf), uterus (ut). The staining pattern is described in the text of the Results section. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Both these antifilarial compounds cause mitochondria mediated apoptosis in *S. cervi* leading to their death [18,19]. The expression of actin remains unaltered in the tissue sections of the aspirin and SK7 treated parasites in comparison to the control. However, a decrease in green fluorescence of PTPL in case of aspirin treated parasites was found. This was very much evident from the high intensity of red fluorescence at the places of overlay of PTPL and F-actin (earlier showing yellowish green fluorescence in the control sections). However, in case of SK7 treated parasites, there was slight variation observed in the expression of PTPL in the coelomyrian cells and no alteration in the expression pattern of F-actin was observed in this case too. It is very much evident from the above results that PTPL expression is altered after drug exposure and independent of the actin expression. The expression of PTPL protein is somehow correlated with the survival of the parasites and therefore might be involved in the persistence and pathogenesis of the parasite. The reduction in expression of PTPL in the parasites might be due to its post translational modification after drug exposure.

This is the first report showing the purification, characterization and role of a FERM-domain-containing phosphatase in any filarial parasite to the best of our knowledge.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Transparency document

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