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ORIGINAL PAPER

# Proteomic profile approach of effect of putrescine depletion over *Trichomonas vaginalis*

María Elizbeth Alvarez-Sánchez[1] · Laura Itzel Quintas-Granados[1] · Laura Isabel Vázquez-Carrillo[1] · Jonathan Puente-Rivera[1] · Alma Villalobos-Osnaya[1] · María Dolores Ponce-Regalado[2] · Minerva Camacho-Nuez[1]

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## Abstract

Infection with *Trichomonas vaginalis* produces a malodorous seropurulent vaginal discharge due to several chemicals, including polyamines. The presence of 1,4-diamino-2-butanone (DAB) reduces the amount of intracellular putrescine by 90%, preventing the cotransport of exogenous spermine. DAB-treated parasites present morphological changes, which are restored by adding exogenous putrescine into the culture medium. However, the effect of polyamines over the trichomonad proteomic profile is unknown. In this study, we used a proteomic approach to analyze the polyamine-depletion and restoration effect by exogenous putrescine on *T. vaginalis* proteome. In the presence of inhibitor DAB, we obtained 369 spots in polyamine-depleted condition and observed 499 spots in the normal culture media. With DAB treatment, the intensity of 43 spots was increased but was found to be reduced in 39 spots, as compared to normal conditions. Interestingly, in DAB-treated parasites restored with a medium with added exogenous putrescine, 472 spots were found, of which 33 were upregulated and 63 were downregulated in protein intensity. Some of these downregulated proteins in DAB-treated parasites are involved in several cellular pathways such as glycolysis, glycolytic fermentation, arginine dihydrolase pathway, redox homeostasis, host cell binding mediated by carbohydrate, chaperone function, and cytoskeletal remodeling. Interestingly, the intensity of some of the proteins was restored by adding exogenous putrescine. In conclusion, the presence of DAB altered the proteomic profile of *T. vaginalis*, resulting in a decrease in the intensity of 130 proteins and an increase in the intensity of 43 proteins that was restored by the addition of putrescine.

**Keywords** *Trichomonas vaginalis* · Putrescine restoration · 2-DE · Mass spectrometry · Proteomic approach

## Introduction

The biologically active polyamines are low molecular weight diamines and triamines, including putrescine, spermidine, and spermine (Agostinelli et al. 2010). These protonated polycations are essential for cell growth, regulating several cellular activities at replication, transcription, translational, and posttranslational levels (Takahashi and Kakehi 2010). Putrescine is a particularly important factor during trichomoniasis, a sexually transmitted infection caused by the protozoan parasite *Trichomonas vaginalis*. This infection is associated with health problems mainly in women, with symptoms such as vaginitis, cervicitis, infertility, low birth weight infants, preterm delivery, and a predisposition to cervical neoplasia (Cotch et al. 1997; El-Shazly et al. 2001; Schwebke and Burgess 2004; Viikki 2000). A malodorous seropurulent vaginal discharge is frequent in this infection possibly due to an increase in vaginal biogenic amines, including the polyamines putrescine, cadaverine, trimethylamine, and thiols (Huang et al. 2014; Nelson et al. 2015; Yeoman et al. 2013). *T. vaginalis* has high intracellular amounts of putrescine (196–38 nmol/mg protein) with a lower amount of spermidine (9.7–3.5 nmol/mg protein) and spermine (1.3–19 nmol/mg protein) (Gillin et al. 1984; North et al. 1986; White et al. 1983; Yarlett and Bacchi 1988). In vaginal fluids, the putrescine concentrations are 4- to 54-fold higher compared to the 45 μM

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✉ María Elizbeth Alvarez-Sánchez
elizbeth2@gmail.com; maria.alvarez@uacm.edu.mx

[1] Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México (UACM), San Lorenzo # 290, Col. Del Valle, 03100 Mexico City, Mexico

[2] Departamento de Clínicas, Centro Universitario de los Altos, Universidad de Guadalajara, Tepatitlán de Morelos, Jalisco, Mexico

concentration present in the supernatant. The physiological functions of polyamines during the trichomoniasis are interesting due to the unusually large amounts of intracellular putrescine produced and excreted via an energy-generating arginine dihydrolase pathway in *T. vaginalis* (Linstead and Cranshaw 1983; Yarlett et al. 1996). The excreted putrescine, synthesized by the trichomonad enzyme ornithine decarboxylase (ODC), is used for the uptake of host spermine via a putrescine-spermine antiporter system (Yarlett and Bacchi 1994). The inhibition of ODC, by 20 mM 1,4-diamino-2-butanone (DAB), causes a 90% reduction in the amount of intracellular putrescine, preventing the cotransport of exogenous spermine (Garcia et al. 2005; Reis et al. 1999). However, DAB-treated parasites maintain their motility while the viability of the trichomonads is restored by adding 40 mM exogenous putrescine into the culture medium. This is due to competition with DAB for transporting spermine into the cell, which results in the restoration of the putrescine-spermine antiporter system (Reis et al. 1999; Yarlett et al. 2000). Aside from the adhesion of the parasite to the host cells (Garcia et al. 2005), polyamines regulate some virulence factors, such as the cysteine proteinase TvCP39 (Carvajal-Gamez et al. 2014) and TvCP65, involved in cytotoxicity toward host cells (Alvarez-Sánchez et al. 2008). In DAB-treated parasites, the proteolytic activity, the TvCP65 protein, and the transcript amount were reduced. Another protein of *T. vaginalis* that is regulated by polyamines is the eukaryotic translation initiation factor (TveIF-5A), the unique only polyamine-dependent protein synthesized as a precursor, which is glycosylated and phosphorylated to produce the mature and functional TveIF-5A (Carvajal-Gamez et al. 2012). Until now, studies have focused on putrescine at molecular level; however, the effect of this cation on the proteomic profile of *T. vaginalis* is still unknown. Here, we report the proteomic changes in *T. vaginalis* due to putrescine depletion by DAB and the restoration with exogenous putrescine.

## Material and methods

### *T. vaginalis* culture

*T. vaginalis* CNCD 147 clinical isolate was grown to the mid-logarithmic phase in Diamond's trypticase-yeast extract-maltose (TYM) medium (pH 6.2), supplemented with 10% (*v*/*v*) heat-inactivated horse serum.

### Putrescine depletion and restoration culture conditions

*T. vaginalis* CNCD 147 parasites were grown in mid-logarithmic 24 h at 37 °C in TYM-serum medium supplemented with 20 mM DAB (Sigma Chemical Co., St. Louis, MO). $1 \times 10^8$ parasites were obtained for 2-DE analysis (samples termed +DAB). Then, for putrescine restoration, the DAB-treated parasites were washed with sterile PBS 1× pH 7.0 and cultured to TYM-serum medium supplemented with 40 mM putrescine (Sigma) (samples termed +DAB/+PUT) or cultured to normal control TYM-serum medium (samples termed +DAB/+CTRL). Additionally, parasites grown in a TYM-serum medium for 24 h at 37 °C were cultured to TYM-serum medium (samples termed CTRL) as a control. Parasites were incubated at 37 °C for 30 min, and all the samples (1 × 108) were collected by centrifugation for 2-DE assays. Parasite viability was measured with the trypan blue (Sigma) exclusion method.

### Protein extraction for 2-DE assays

Parasites ($1 \times 10^8$) were washed with PBS 1× pH 7.0 and lysed by sonication (30 s on/off ice for 3 min) in the same buffer supplemented with Pierce Protease Inhibitor Tablets (Thermo Scientifc, Waltham, MA, USA) according to the manufacturer's instructions. Samples were centrifuged 16,000*g* at 4 °C for 5 min to obtain the soluble fraction and were precipitated with TCA 10%. Finally, the pellets were resuspended in rehydration buffer (7 M urea, 4% CHAPS, 70 mM DTT, 2% IPG buffer pH 4–7, trace bromophenol blue; Bio-Rad, Hercules, CA, USA) and quantified using the Coomassie (Bradford) Protein Assay Kit (Thermo Scientific) according to the manufacturer's instruction.

### Two-dimensional electrophoresis assays

Exactly 200 μg of the total protein of *T. vaginalis* treated in normal, depleted, and restored putrescine conditions were applied to an IPG strip (17 cm, pH 4–7 linear; Bio-Rad) for passive rehydration for 12 h in a Protean IEF system (Bio-Rad) as follows: step one, gradient from 1 to 500 V over 60 min; step two, 1000 V over 60 min; step three, 6000 V over 110 min; and step four, 6000 V to 20,000 Vh. Before the second dimension, samples were reduced (10 mg/ml DTT) and alkylated (25 mg/ml iodoacetamide) stepwise, 15 min for each step, in equilibration buffer (6 M urea, 2% SDS, 300 mM Tris-Cl pH 8.8, 20% glycerol, and 0.002% bromophenol blue) at 25 °C. Equilibrated IPG strips were separated on 12% SDS-PAGE gels and stained with Sypro Ruby protein (Bio-Rad), according to the manufacturer. Finally, gels were documented in Gel Doc EQ (Bio-Rad). Image analysis was performed using the pDQuest (Bio-Rad) and the Melanie 2D gel analysis 7.05 software. The functional classification of *T. vaginalis* proteome was performed according to the Gene Ontology index (http://www.geneontology.org/).

### LC-ESI-QUAD-TOF

The MS analysis of each fraction obtained from the offline separation steps was carried out using a QUAD-TOF mass spectrometer (Waters Corporations, Milford, MA) equipped with a nanoelectrospray ion source (NanoSpray II). The instrument was coupled online with a nanoAcquity Ultra Performance LC system (Waters Corporations, Milford, MA, USA). Samples were desalted by injection onto a Symmetry C18 UPLC trapping capillary column (180 μm × 20 mm, Waters Corporations) and washed with 0.1% formic acid in 100% MilliQ water at a flow rate of 15 ml/min. After 3 min, the trap column was switched in line with a capillary analytical column. Peptides were separated on an ethylene-bridged hybrid, C18 UPL column (75 μm × 100 mm, Waters Corporations) using a linear gradient of 2–70% acetonitrile and 0.1% formic acid over a 60-min period at a flow rate of 0.25 ml/min. Spectra were acquired in automated mode using information-dependent acquisition (IDA). Precursor ions were selected in Q1 using the enhanced MS mode with a scan range of m/z 400–1500 and 4000 amu/s. Selected ions were subjected to an enhanced resolution scan at a low speed of 250 amu/s over a narrow (30 amu) mass range to an enhanced product ion scan (MS/MS). Precursor ions were fragmented by collision-activated dissociation (CAD) in a Q2 collision cell using rolling collision energy. The generated fragment ions were captured and mass-analyzed in a Q3 linear ion trap.

### Search parameters and acceptance criteria

Name of peak list generating software: Mascot Distiller v2.1. Name of the search engine and release version (number or date): MASCOT server (Matrix Science, London, UK, available at http://www.matrixscience.com, version 2.2; Matrixscience, London, UK). Name of sequence database/spectral library searched and release version/date: NCBI nr 2011.07.09 (14,652,852 sequences; 5,012,444,178 residues). Number of entries in the database (or subset of database) actually searched: 379307 sequences. The genome of *T. vaginalis* is available at http://trichdb.org/trichdb/ with the following parameters used: default parameters of Mascot, enzyme specificity: trypsin, maximum missed cleavages: 1, fixed modifications: Carbamidomethyl (C) (+57.021 Da at cysteine residue), variable modifications: deamidated (+1 Da at cysteine residue), and methionine oxidation (+15.995 Da at methionine residue), precursor ion mass tolerance: 100 ppm, fragment ion mass tolerance: 0.6 Da.

### Light microscopy

*T. vaginalis* was grown at 37 °C in the conditions described above (CTRL, +DAB. +DAB/+PUT, +DAB/CTRL), and the parasite morphologies were determined by differential interference contrast microscopy (DIC) through which the culture was observed at × 40 magnification. Counting of $1 \times 10^4$ parasites per assay was performed by triplicates of each condition after 30 min of incubation with a hemocytometer, and viability was tested by using trypan blue exclusion.

## Results

In the proteome profile from parasites grown in control culture condition, 499 spots were obtained, the most abundant of which were observed from 20 to 75 kDa and pH near 7.0 (Fig. 1a). Nevertheless, when the parasite was grown with inhibitor DAB presence, the proteomic profile was modified. We identified 369 spots showing a similar pattern of protein spot distribution with respect to molecular weight and pI ranges (Fig. 1c), 130 spots less as compared with the control after the treatment of parasites with DAB (Fig. 2). We found that 82 out of the 369 spots in DAB condition were intensity-regulated and half of them (43) were threefold upregulated. In contrast, the intensity of 39 spots was threefold downregulated (Table 1) while 287 spots may have been unaffected by treatment with DAB (Fig. 2).

The DAB inhibitor has an effect in the proteome of *T. vaginalis*; therefore, we restored polyamines present in the medium by adding exogenous putrescine, detecting 472 spots (Fig. 1c) when compared with the 369 spots detected in DAB-treated parasites. A total of 103 spots were restored in the presence of exogenous putrescine (Fig. 2); however, the intensity in 27 spots was not restored in this condition compared with the normal condition (Fig. 2) while 96 spots were now regulated: 63 spots were threefold downregulated and 33, upregulated (Table 1). As a control, parasites in DAB-treated condition were cultured in control media, where 354 protein spots were obtained, of which 37 increased and 44 decreased, showing a similar number of proteins from parasites grown in DAB (Fig. 1d). Therefore, the presence of exogenous putrescine increased the number of spots (Table 1). Some spots were conserved between the DAB and putrescine-restored parasite treatment, so we selected 15 conserved protein spots to be identified by mass spectrometry. The peptides identified corresponded to proteins related to diverse cellular functions in the parasite, such as energy catabolism enzymes, protein folding, and structure cytoskeleton protein (Supplementary Table 1).

Among the parasites grown in DAB conditions, we identified downregulated proteins with functions in glycolytic fermentation (L-lactate deshydrogenase), arginine dihydrolase pathway (carbamate kinase), redox homeostasis mechanism (thioredoxin reductase), and carbohydrate-binding function (lectin repeat protein). Contrastingly, the identified peptides correspond to proteins of glycolytic pathway, as fructose

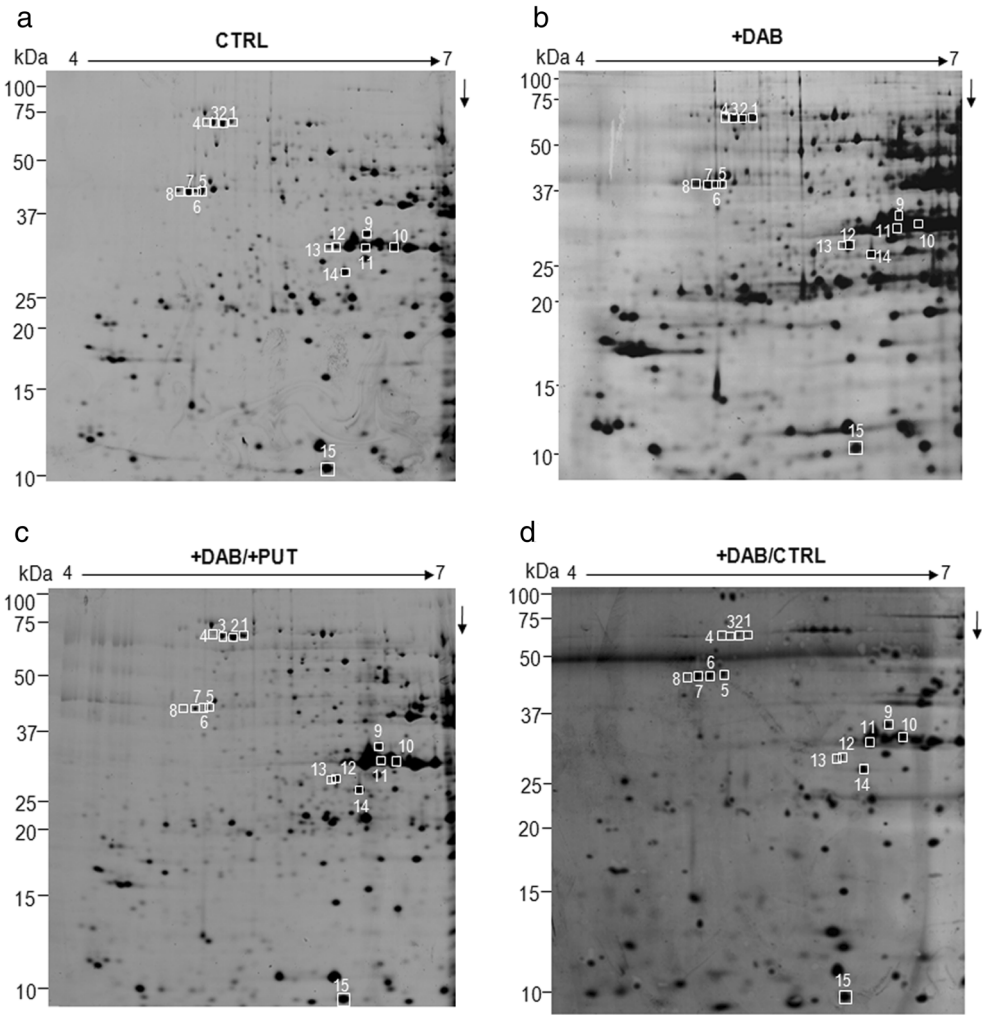


**Fig. 1 Proteomic approach to putrescine depletion/restoration effect on *T. vaginalis*.** Identification of proteins by 2-DE assays of trichomonad total protein extract from control culture conditions (CTRL) (**a**), DAB-treated trichomonads (+DAB) (**b**), DAB-treated trichomonads treated with exogenous putrescine (+DAB/+PUT) (**c**), and DAB-treated trichomonads cultured in control medium (+DAB/+CTRL) and in IPG pH linear gradient 4–7 separated in 10% SDS-PAGE. White squares indicate the spots identified by MS/MS. Rightwards arrow indicates direction of IEF. Downwards arrow indicates direction of the SDS–PAGE by molecular weight. kDa, molecular weight marker (Bio-Rad). Three independent repeats were performed for each experiment, with similar results

1,6-bisphosphate aldolase, in protein-folding function as 70 kDa cytoplasmic heat shock protein or cytoskeletal protein

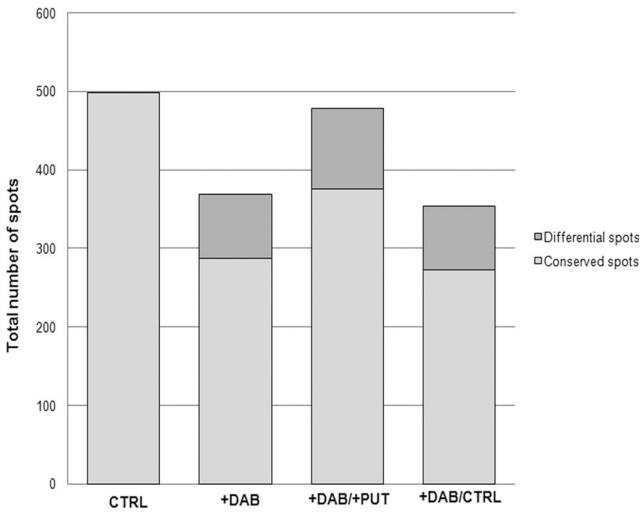


**Fig. 2 Distribution of spots in proteomics approach control, DAB, and exogenous putrescine-treated parasites.** The bar indicates the quantity of spots obtained in the conditions. Black bars, conserved proteins between conditions. Grey bars, differential spots

(Fig. 3). A decrease in the intensity of the identified TvHSP70 (gene bank access number gi|1943757) in the DAB-treated condition (Fig. 4a, +DAB, spot 1) was detected, as compared to control conditions (Fig. 4a, CTRL, spot 1). Nevertheless, the intensity of this chaperone was restored by adding exogenous putrescine (Fig. 4a, spot 1, +DAB/+PUT). Interestingly, the intensity level was not restored by the culture of parasites under control condition (Fig. 4a, spot 1, +DAB/CTRL). A decrease of intensity during the DAB treatment (Fig. 4, spots 2–4, +DAB) was also found in the other TvHSP70 isoform identified (gi|123468246) (Supplementary Table 1) when

**Table 1** The effect of putrescine on the proteome of *T. vaginalis*

| Condition | Total spots | Threefold up* | Threefold down* |
|---|---|---|---|
| CTRL | 499 | – | – |
| +DAB | 369 | 43 | 39 |
| +DAB/+PUT | 472 | 33 | 63 |
| +DAB/CTRL | 354 | 37 | 44 |

*The number of fold up or down spots was obtained by comparison with the trichomonads grown in control culture (CTRL)

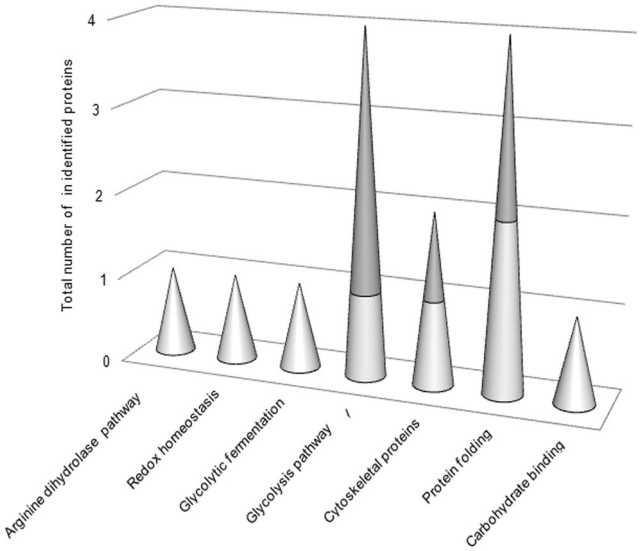


**Fig. 3 Analysis of proteomics approaches of putrescine-regulated proteins in *T. vaginalis*.** Identified proteins with a regulated intensity in putrescine-depleted conditions involved in several biological processes as metabolism (six proteins), redox homeostasis (one protein), and carbohydrate-binding (one protein) functions, while others are chaperons (four proteins) or cytoskeletal proteins (two proteins)

compared to the control (Fig. 4, spots 2–4, CTRL). However, one spot intensity of this isoform was not restored by added exogenous putrescine (Fig. 4, spot 4, +DAB/+PUT) or by the control condition (Fig. 4, spot 4, +DAB/CTRL).

This result suggests a modulating effect of putrescine over the expression and subsequent production of a possible specific isoform of HSP70, given that the presence of putrescine leads to an increase in protein intensity. The analysis of peptide-identified groups showed that both correspond to the same accession number protein (TVAG_151220) that in turn corresponds to gene *tvhsp70* instead of TvHSP70 putative (TVAG_092490), according to the ESTs analysis performed by Figueroa-Angulo et al. (2015). In addition, we also observed some differences in the intensity of *T. vaginalis* actin (TvACT) (Fig. 4b). Spot numbers 5 and 7 were intensity upregulated by DAB treatment (Fig. 4b) and corresponded to access number AAB05803.1 and TVAG_200190, respectively, indicating different proteins (Fig. 4b, spots 5 and 7, +DAB). However, adding exogenous putrescine was not sufficient to restore the intensity of all TvACT proteins, including the possible isoforms (Fig. 4b, +DAB/+PUT). There was a decrease in spot intensity among fructose-1,6-bisphosphate aldolase (TvFBA, TVAG_300000), L-lactate dehydrogenase (Tv-L-LDH, TVAG_171100) (Fig. 4c, spot 9), and triosephosphate isomerase (TvTIM, TVAG_096350) (Fig. 4d, spot 12) in DAB-treated parasites (+DAB), compared with the trichomonads cultured in control conditions (Fig. 4, CTRL). Nevertheless, the intensity

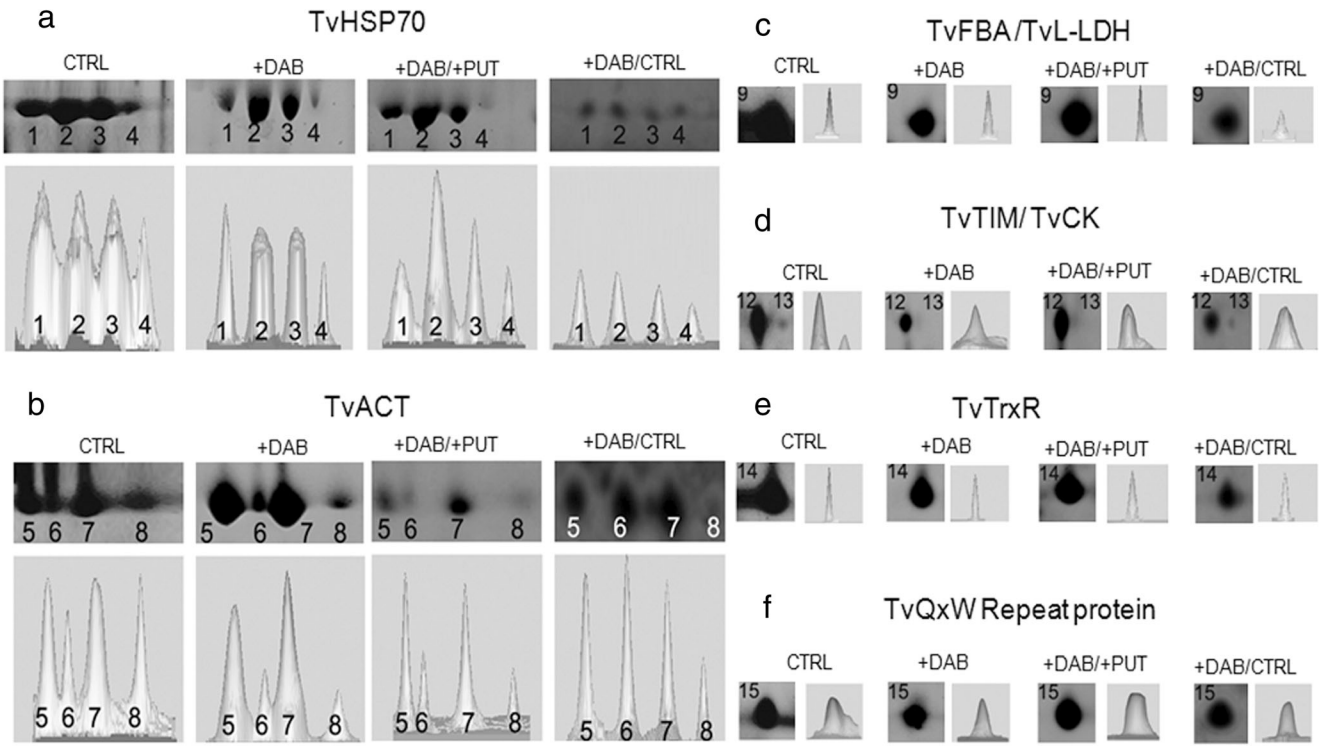


**Fig. 4 Putrescine effect over the intensity of trichomonad proteins in proteomics approach.** Close-up images obtained by PDQuest analysis from the proteomes described in Figures 1 and 2. Spots 1 to 4 correspond to 70 kDa cytoplasmic heat shock protein (TvHSP70). Spots 5 to 8 possibly correspond to different actin (TvACT) isoform species. Spot 9 corresponds to fructose-1,6 bisphosphate aldolase. Spot 12 corresponds to triosephosphate isomerase. Spot 13 corresponds to carbamate kinase. Spot 14 corresponds to thioredoxin reductase, and spot 15 corresponds to QxW lectin repeat family protein

was restored after adding exogenous putrescine (Fig. 4, +DAB/+PUT). This result also suggests a possible correlation between the polyamine metabolism and the aerobic/anaerobic glycolytic pathway.

In addition, we identified the carbamate kinase (TvCK, TVAG_261970) to be an enzyme involved in the arginine metabolic process, with a reduced intensity in its identified spot (Fig. 4e, spot 13, +DAB), compared against the control condition (Fig. 4, spot 13, CTRL). Interestingly, the intensity of the spot was not restored after adding exogenous putrescine (Fig. 4, spot 13, +DAB/+PUT).

Additionally, the DAB treatment (Fig. 4, spots 13 and 14, +DAB) did not affect the intensity of thioredoxin reductase (TvTrxR, TVAG_474980A) (Fig. 4f, spot 14) or QxW lectin repeat family protein (TVAG_261950A) (Fig. 4f, spot 15). Both proteins are constitutive in *T. vaginalis*: the first participates in the reduction of reactive oxygen species (ROS) while the second is involved in carbohydrate-binding function given that the QxW domain is implicated in parasite adherence to host cells based on their carbohydrate specificities. The light microscopy observations revealed that the trichomonads collected from DAB-treated conditions (+DAB) show an important change in shape, becoming swarming rounded aggregates with a pseudocyst-like form (Fig. 5b). In turn, these depleted parasites received an exogenous putrescine treatment (+PUT) after which the morphology of the parasites was restored (Fig. 5c) to the typical ovoid form observed in control condition (Fig. 5a). A similar pattern was observed in the morphology and aggregation of parasites grown in DAB conditions cultured into control media (+DAB/CTRL).

## Discussion

Polyamines are important cations known for their ability to stabilize DNA, RNA, and proteins, regulating several processes including proliferation, differentiation, and cell division (Igarashi and Kashiwagi 2010; Jänne et al. 2004) and are present in the female microenvironment (Nelson et al. 2015). However, the effects of the cations over the proteomic profile of *T. vaginalis* have yet to be widely characterized. The proteomic map of this sexually transmitted parasite has already been reported (De Jesus et al. 2007a, b), with a focus on the possible microenvironmental effects of $Fe^{2+}$ or $Zn^{2+}$

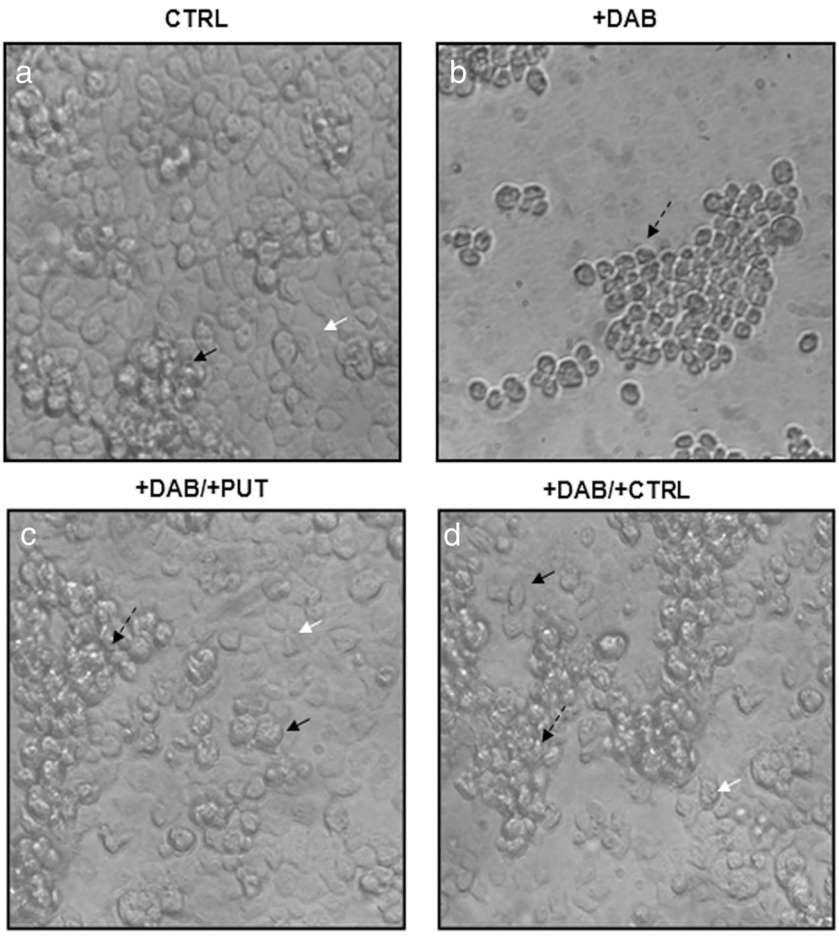


**Fig. 5 The effect of putrescine depletion/restoration in the morphology of *T. vaginalis*.** (**a**) Interferential contrast microscopy (DIC) of *T. vaginalis* CNCD147, showing typical ovoid (full black arrow) and adherent (full white arrow) morphology in control TYM-serum medium. (**b**) Parasites cultured in a DAB-treated TYM-serum medium showing mainly a spherical shape and swarming (dotted arrow) and non-adherent parasites. (**c**) Parasites treated with DAB incubated with exogenous putrescine showing a more less spherical shape and swarming (dotted arrow) and more ovoid (full black arrow) and adherent (full white arrow) morphology. (**d**) Parasites treated with DAB incubated with a TYM-serum medium showing a spherical shape and swarming (dotted arrow) and more ovoid (full black arrow) and adherent (full white arrow) morphology. Parasites were observed in light microscopy in × 40 objective

concentrations over the protein differential expression (Vazquez-Carrillo et al. 2011).

In this report, 499 spots were identified in the proteomic profile of parasites grown in normal culture media. In parasites grown in polyamine-depleted conditions treated with DAB, an ODC inhibitor, only 369 spots were detected, which is an important reduction in the total number of spots. After treatment with DAB in *T. vaginalis*, we determined the growth arrest of parasites without compromising their viability (Yarlett and Bacchi 1988) and a reduction in the levels of trichomonal cytotoxicity. These showed the dependence on the polyamine metabolism to correctly maintain cellular processes and adapt to future adverse conditions (Pegg 2009). When exogenous putrescine was added to the polyamine-depleted trichomonads culture, the total increase in the number of protein spots, from 369 to 472, shows that the effect of putrescine depletion on the trichomonads proteome is reverted by the exogenous putrescine. The effects of exogenous application of polyamines (putrescine, spermidine, or spermine) at different concentrations have been demonstrated to confer enhanced tolerance to various stress conditions in cell plants (Duan et al. 2008). The polyamine depletion by inhibitors of ODC in murine B cells induced apoptosis; however, the effect was reverted by the addition of exogenous polyamines (Nitta et al. 2002). During trichomoniasis, the fluctuations in $Fe^{2+}$ concentrations in the vagina might modulate the expression and functions of several proteins; for instance, in $Fe^{2+}$-depleted conditions, the parasite upregulated the expression of two 70 kDa heat shock proteins (TvHSP70) and one actin (TvACT). In contrast, there is a significant decrease in the expression of pyruvate:ferredoxin oxidoreductase (PFO) and ferredoxin in the presence of the cation (De Jesus et al. 2007b).

In healthy women, the levels of putrescine and diamines in vaginal secretions are undetectable, but during the trichomoniasis, the putrescine levels increase (> 2 mM) in the vaginal fluids, suggesting that the parasite also produces this polyamine during the infection (Yarlett and Bacchi 1988, Alvarez-Sánchez et al. 2017). These biological polyamines could also participate in other cellular functions in the parasite, as reported in hepatitis C virus (Korovina et al. 2012). The TvHSP70 isoforms participate in different cellular functions, as protein folding, during their maturation (Kampinga and Craig 2010).

A decrease in the abundance of the four isoforms of TvHSP70 was detected in *T. vaginalis* after DAB treatment and once the addition of exogenous putrescine restored the intensity of three spots of those isoforms. These spots were identified as the same specific cytoplasmic TvHSP70, according to the amino acid sequence with the ID TVAG_044510 (Figueroa-Angulo et al. 2015). We were unable to identify one of them by MS; this might be explained because there are 11 highly conserved *tvhsp70* genes in *T. vaginalis* (Carlton et al. 2007).

In addition, the parasite expresses multiple *tvact* genes, which indicates a possible advantage in its adaptation to different environments (Bricheux and Brugerolle 1997), including cation presence as $Fe^{2+}$ (De Jesus et al. 2007a, b) and polyamines during the adherence event of the parasite to host cells (Garcia et al. 2005). With this background, we cannot discard the possibility that a specific isoform of TvHSP70 plays a specific role in polyamine metabolism, dismissed by inhibition by DAB and unable to be restored in quantity by the exogenous putrescine addition. At least four possible conserved TvACT isoforms were also detected in the control and polyamine-depleted condition. Actin constitutes about 10% of the total proteins and is involved in pseudopodia formation in the parasite (Müller 1990), similar to what has been found in eukaryotic mammalian cells (Ganem 1997).

The arginine dihydrolase pathway (ADH) in trichomonads has an analogous function to the urea cycle described in other eukaryotes, catalyzing the conversion of arginine to ornithine and ammonia via the enzymes arginine deiminase (TvADI), catabolic ornithine carbamoyltransferase (TvOCT), and carbamate kinase (TvCK) (Morada et al. 2011). Interestingly, our study found that the abundance of TvCK decreased under polyamine-depleted conditions. This enzyme is involved in the last step of the ADH pathway, converting carbamoyl phosphate and ADP into ammonia, carbon dioxide, and ATP. The second step of the pathway involves the degradation of L-citrulline to carbamoyl phosphate and L-ornithine, using TvOCT (Linstead and Cranshaw 1983).

*T. vaginalis* is microaerophillic and anaerobic (Shiflett and Johnson 2010) and expresses thioredoxin reductase (TvTrxR), which functions together with thioredoxin and thioredoxin peroxidase to eliminate potentially damaging oxidants (Coombs et al. 2004). Interestingly, in polyamine-depleted conditions, the spot intensity of TvTrxR abundance was not changed when compared with the control condition, suggesting that in polyamine presence and depletion, some oxidant compounds are formed, which could affect the parasite. It has been described that in *Escherichia coli*, hydroxyl radicals, hydrogen peroxide, and superoxide anions are considered ROS, which are produced in the presence of antibiotics. The cells respond to oxidative stress with a two-to-threefold increase in cell polyamine production, as a consequence of the upregulation of ODC. When ROS are reduced, polyamine accumulation is also substantially diminished or even completely depleted, suggesting that polyamines reduce oxidative stress in *E. coli* (Tkachenko et al. 2012).

In *T. vaginalis*, the absence of polyamines might generate oxidative stress that the parasite might respond to by activating its major antioxidant non-modulate defense mechanism, TvTrxR. The cause-effect relationship between polyamines and oxidative stress has also been observed in plants that become tolerant to stress after the addition of exogenous polyamines (Duan et al. 2008). In *T. cruzi*, redox imbalance contributes to DAB cytotoxicity through pro-oxidant properties,

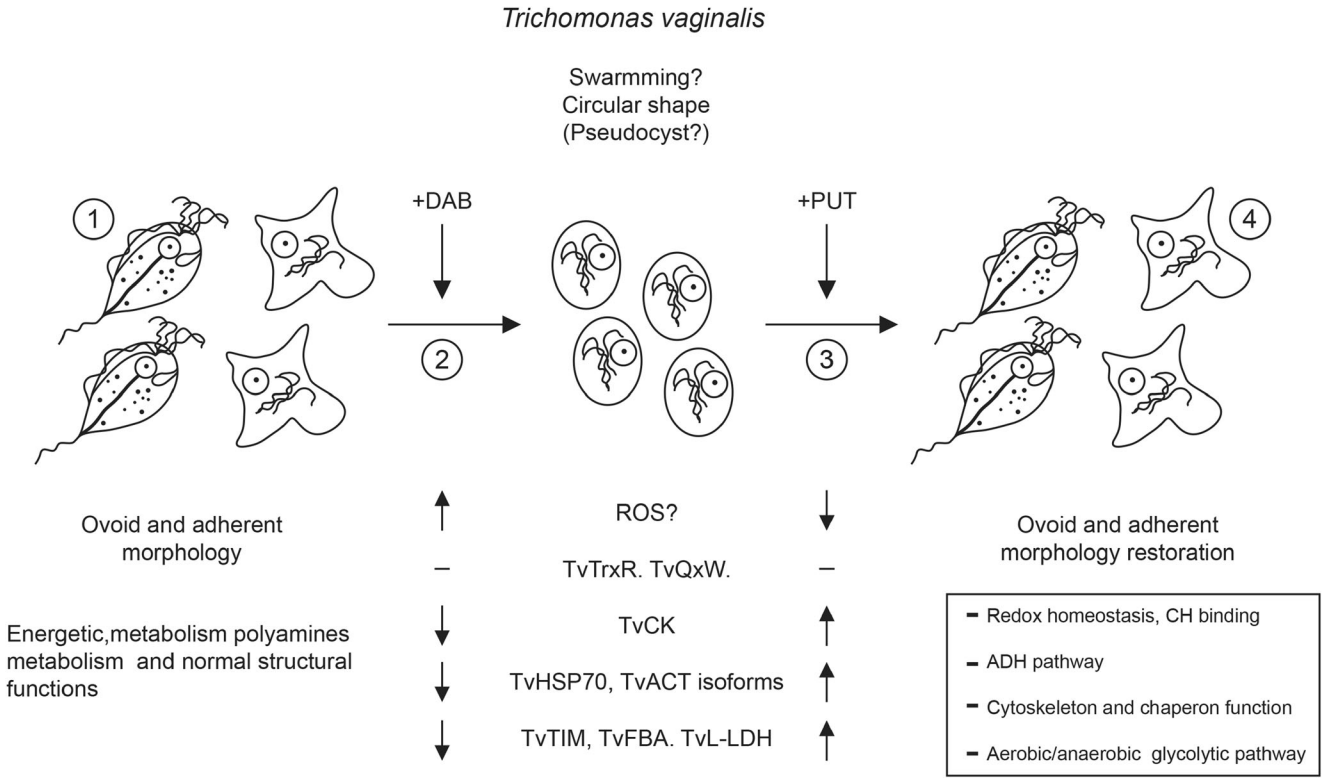


**Fig. 6 Proposed model of putrescine depletion (+DAB) and subsequent restoration (+PUT) effects over protein abundance in *T. vaginalis*.** (1) In normal putrescine conditions, the parasites present normal energetic and polyamines metabolism. (2) In the presence of DAB inhibitor, a possible ROS increase is present. Proteins of PA metabolism mediated by ADH (TvCK) and glycolytic metabolism pathways (TvTIM, TvFBA, and TvL-LDH) and isoforms of TvHSP70 are also decreased. Also, a morphology change to possibly swarming and pseudocyst shape mediated by an isoform of TvACT are observed. (3) Exogenous putrescine addition (+PUT) revealed a possible PA and a glycolytic metabolic shift that decreased ROS suitable for the survival and accurate protein folding and TvACT isoform to ovoid and adherent restoration

changes in morphology, thiol redox imbalance, and increased superoxide dismutase TcSOD activity (Soares et al. 2012). Moreover, TvTrxR has been proposed as a possible therapeutic target for trichomoniasis treatment (Hopper et al. 2016).

The abundance of glycolytic enzymes, TvFBA, and one of the TvTIM2 was reduced in the presence of DAB; however, their levels were restored by the addition of exogenous putrescine. TIM protein is involved in the reversible interconversion of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) in Embden-Meyerhof-Parnas, gluconeogenesis, and the pentose phosphate pathways (Wierenga et al. 2010); it is overexpressed in high-glucose conditions and is capable of interacting with ECM proteins (Miranda-Ozuna et al. 2016). The TvFBA of the parasite belongs to Class II of FBA proteins (Sánchez et al. 2002), involved in the fourth step of glycolysis to obtain bisphosphate from dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (Marsh and Lebherz 1992). In neuroblastome cells, a glycolysis inhibition trigger signal to decrease both ODC expression and polyamine levels is observed; this is the first reported relationship between a glycolytic pathway and polyamines levels (Ruiz-Pérez et al. 2015).

Moreover, the TvL-LDH reported here is a different protein from the upregulated LDH found in glucose restriction (TVAG_381310) (Huang et al. 2014).

With the regulation of these enzymatic proteins, we hypothesize that *T. vaginalis* has a possible mechanism of negative regulation of glycolytic enzymes mediated by polyamines and restored by exogenous protein addition. The effect of polyamines on the morphology *T. vaginalis* was also observed as a change in the shape of the parasites, from an ovoid into a pseudocyst in presence of DAB, and a reduced adhesive property. The parasite adhesion is multifactorial, mediated by several proteins (Figueroa-Angulo et al. 2012).

The pseudocysts can be induced in vitro upon exposure of trophozoites to cold and stressors (Pereira-Neves et al. 2003), now including polyamine depletion in the medium. A hypothetical protein identified in this work as a repeat QxW family protein, which was not affected in its intensity by putrescine depletion, suggests the participation of lectin domain proteins to host adhesion in a non-specific fashion more than the adhesion mediated by the virulence factor proteins in microenvironmental conditions of the parasite (Hirt 2013).

Finally, it has been hypothesized that polyamines interact with nucleic acids and nucleoprotein complexes involved in

the regulation of diverse cellular processes, including DNA replication, transcription, mRNA processing, and translation (Korovina et al. 2012). To this day, these mechanisms have not been studied in the parasite.

In conclusion, putrescine is an important cation with a possible role as modulator of the abundance of some proteins that participate in several events as morphological variation, protein folding, stress conditions, and metabolic processes (i.e., glycolytic and ADH pathways) in *T. vaginalis*. Inhibition by DAB and the effect over the *T. vaginalis* proteome might be reverted by the addition of exogenous putrescine (Fig. 6). The high amount of putrescine found in vivo might be explained by the role of this cation in the female infection by *T. vaginalis*.

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