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# Effect of the sesquiterpene lactone incomptine A in the energy metabolism of *Entamoeba histolytica*

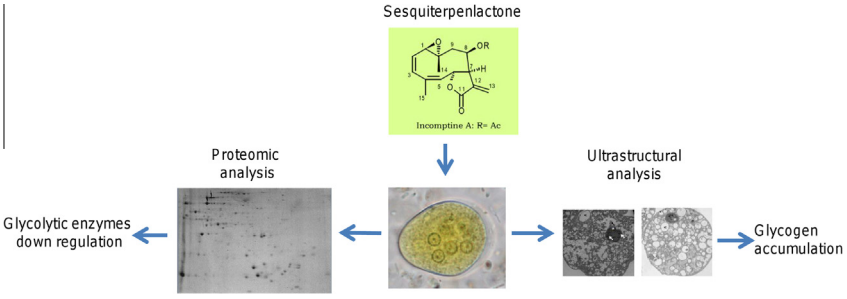
José Velázquez-Domínguez [a], Laurence A. Marchat [a,b], Cesar López-Camarillo [c], Guillermo Mendoza-Hernández [d], Esther Sánchez-Espíndola [e], Fernando Calzada [f], Alfredo Ortega-Hernández [g], Virginia Sánchez-Monroy [h], Esther Ramírez-Moreno [a,b,*]

[a] *Posgrado en Biomedicina Molecular, ENMyH, Instituto Politécnico Nacional, México City 07320, Mexico*
[b] *Doctorado en Biotecnología, ENMyH, Instituto Politécnico Nacional, México City 07320, Mexico*
[c] *Programa de Ciencias Genómicas, Universidad Autónoma de la Ciudad de México, México City 03100, Mexico*
[d] *Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México City 07320, Mexico*
[e] *Central de Microscopía, ENCB, Instituto Politécnico Nacional, México City 11340, Mexico*
[f] *Unidad de Investigación Médica en Farmacología, Centro Médico Nacional Siglo XXI, México City 06725, Mexico*
[g] *Instituto de Química, Universidad Nacional Autónoma de México, México City 04510, Mexico*
[h] *Laboratorio Multidisciplinario de Investigación, Escuela Militar de Graduados de Sanidad, Universidad del Ejército y Fuerza Aérea, México City 11620, Mexico*

## HIGHLIGHTS

- We evaluated the effect of incomptine A on *E. histolytica* protein expression.
- 21 proteins were differentially expressed, including three glycolytic enzymes.
- Incomptine A also induced an increased number of glycogen granules.
- Incomptine A could act through alteration of *E. histolytica* energy metabolism.

## GRAPHICAL ABSTRACT

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## ABSTRACT

*Entamoeba histolytica* is the causative agent of human amoebiasis, which mainly affects developing countries. Although several drugs are effective against *E. histolytica* trophozoites, the control of amoebiasis requires the development of new and better alternative therapies. Medicinal plants have been the source of new molecules with remarkable antiprotozoal activity. Incomptine A isolated from *Decachaeta incompta* leaves, is a sesquiterpene lactone of the heliangolide type which has the major *in vitro* activity against *E. histolytica* trophozoites. However the molecular mechanisms involved in its antiprotozoal activity are still unknown. Using a proteomic approach based on two-dimensional gel electrophoresis and mass spectrometry (ESI-MS/MS) analysis, we evidenced that 21 *E. histolytica* proteins were differentially expressed in response to incomptine A treatment. Notably, three glycolytic enzymes, namely enolase, pyruvate:ferredoxin oxidoreductase and fructose-1,6-biphosphate aldolase, were down-regulated. Moreover, ultrastructural analysis of trophozoites through electronic microscopy showed an increased number of glycogen granules. Taken together, our data suggested that incomptine A could affect *E. histolytica* growth through alteration of its energy metabolism.



## 1. Introduction

*Entamoeba histolytica* is the etiological agent of human amoebiasis. About 10% of the world's population is affected by amoebiasis

*Abbreviation:* STL, sesquiterpene lactone.

* Corresponding author. Address: Laboratorio de Biomedicina Molecular 2, Escuela Nacional de Medicina y Homeopatía-IPN, Guillermo Massieu Helguera No. 239, La Escalera, Ticoman, México City C.P. 07320, Mexico. Fax: +52 57 29 60 00x55538.
*E-mail address:* estherramirezmoreno@yahoo.com (E. Ramírez-Moreno).

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and approximately 40,000–100,000 deaths per year are related to this protozoan, mainly in developing countries (Stanley, 1996, 2003; Fotedar et al., 2007). Although metronidazole and other nitroimidazole compounds are effective for the treatment of invasive amoebiasis, they are less effective eliminating parasites located in the intestinal lumen. In addition, side effects and drug resistance have been described in patients treated with these anti-amoebic agents (Samarawickrema et al., 1997; Hanna et al., 2000). Therefore, new and better alternative therapies are required to control amoebiasis.

Over the last century, medicinal plants have been the source of new molecules with remarkable antiprotozoal activity. The therapeutic properties of medicinal plants are generally attributed to their secondary metabolites, such as sesquiterpenlactones (STL), which are found almost exclusively in species of the Asteracea family (Zhang et al., 2005; Schmidt, 2006; Merfort, 2011). STL are stable C-15 terpenoids compounds that can be divided in four groups: germacranolids, eudesmanolids, guaianolides and pseudoguaianolids (Zhang et al., 2005). STL represent a homogeneous group of secondary metabolites that display a great diversity and an enormously broad spectrum of biological activities. Recently, many STL have been shown to exhibit interesting antiprotozoal activities against *Trypanosoma cruzi* (Brengio et al., 2000; Sülsen et al., 2011), *Plasmodium falciparum* (Pillay et al., 2007; Moon, 2007; Maas et al., 2011), *Giardia intestinalis* (Mena-Rejón et al., 2007) and *Leishmania sp*. (Tiuman et al., 2005). Notably, the discovery of the antimalarial properties of artemisinin, a STL isolated from the Chinese herb *Artemisia annua*, has been a major breakthrough in the field of parasitic diseases and has prompted the investigation of these kinds of compounds (Sülsen et al., 2011).

*Decachaeta incompta* King and Robinson is an herbaceous plant of the Asteraceae family that is commonly used in the local traditional medicine from the state of Oaxaca, Mexico to treat diarrhea and dysentery. The analysis of the dichloromethane extract from *D. incompta* leaves allowed the identification of two STL of the heliangolide type, namely incomptines A and B. Incomptine A had the major *in vitro* activity against *E. histolytica* trophozoites with an $IC_{50}$ value of 2.6 μg/mL, which is in the range of the $IC_{50}$ value of emetine ($IC_{50}$ 1.05 μg/mL), a known amoebicidal drug (Calzada et al., 2009). Recently, two additional heliangolides were isolated from dichloromethane extract of *D. incompta* leaves, incomptines C and D, which showed a lower effect against *E. histolytica* trophozoites as compared with incomptine A, but were more active than incomptine B. Comparative analysis of phytochemical profiles suggested the relevance of the diene and acetate group in C-8 of the germanacrane rings for antiprotozoal activity of incomptine A (Bautista et al., 2012). In order to gain insights into the molecular mechanisms responsible for the antiparasitic activity of incomptine A, we performed here a proteomic study to evidence changes in global protein expression in *E. histolytica* trophozoites treated with incomptine A.

## 2. Materials and methods

### 2.1. Incomptine A purification

Incomptine A was isolated from the aerial parts of *D. incompta* (syn.: *Eupatorium incomptum*; Asteraceae) collected in the State of Oaxaca, Mexico. The plant was identified by Jose Luis Villaseñor Rios, from the Herbarium MEXU of Instituto de Biología UNAM (voucher No: MEXU 1198517). The extraction and isolation procedure was performed according to the protocol previously described by Calzada et al., 2009. Incomptine A was identified by comparison (NMR and TLC) with authentic samples and had a high purity (>99.99%). Dimethyl sulfoxide (DMSO) was used as dissolvent.

### 2.2. E. histolytica trophozoites culture

*E. histolytica* trophozoites, HM1-IMSS strain, were axenically grown in complete TYI-S-33 medium, supplemented with 10% bovine serum (Diamond et al., 1978) and harvested in the log phase of growth. *E. histolytica* trophozoites were incubated for 48 h at 37 °C in the presence of 2.6 μg/mL ($IC_{50}$) incomptine A dissolved in DMSO as described (Calzada et al., 2009). Trophozoites grown in the absence of incomptine A and in the presence of DMSO were included as control groups in all experiments.

### 2.3. Protein extraction

Trophozoites were lysed in 100 mM Tris (500 μl) in the presence of 40 μl/mL Complete proteases inhibitor cocktail (Roche). Samples were centrifuged at 15,000×*g* (14,000 rpm) for 5 min at 4 °C, supernatant was retrieved and cleaned using the ReadyPrep 2D Clean Up kit (Bio-Rad) according to the manufacturer's protocol. Then, protein pellets were dissolved in 100 μl sample buffer (8 M urea, 4% CHAPS, and 80 mM dithiotreitol [DTT]) and protein concentration was determined using the Bradford method. Protein integrity was also assessed by 10% SDS–PAGE and Coomassie blue staining.

### 2.4. Two-dimensional electrophoresis (2-DE)

Protein samples (350 μg) obtained from *E. histolytica* trophozoites grown with or without incomptine A were mixed with 200 μl rehydration solution (8 M urea, 4% CHAPS, 2% ampholines pH 3–10, 0.002% bromophenol blue, 80 mM DTT), and loaded onto 11 cm ReadyStrip IPG strips (linear pH gradient 4.0–7.0, Bio-Rad). IPG strips were passively hydrated for 12 h. Then, proteins were isoelectrically focused using the Protean IEF Cell (Bio-Rad) in four steps: an initial gradient from 1 to 250 V for 30 min, followed by a gradual increase from 250 to 8000 V for 4 h, for a total of 50,000 V/h. Finally, a hold step at 100 V was applied. Next, samples were successively reduced with 2% DTT and alkylated with 2.5% iodoacetamide in equilibrium solution (6 M urea, 2% SDS, 0.375 M Tris–HCl pH 8.8, 20% glycerol) for 10 min at room temperature. For the second dimension analysis, samples were separated on 13% SDS–PAGE at 200 V in a vertical electrophoresis system using a 80 V pre-running step for 20 min followed by a 200 V running step until samples reach the bottom of gel. After electrophoresis, gels were fixed with 50% methanol/7% acetic acid solution, and overnight stained with Sypro-Ruby dye (Invitrogen) according to manufacturer's protocols. Three independent experiments were performed to assess the reproducibility of the assay.

### 2.5. Detection of differentially expressed proteins

Images from 2-D gels were documented in a FLA-5100 Fuji Film scanner and adjusted using the Multigauge software. PD-Quest Advanced software version 8.0 (Bio-Rad) was used for comparative analyses of images corresponding to proteins obtained from incomptine A treated and untreated trophozoites. For 2-D spots selection, images of gels from treated and untreated trophozoites were used to create a match-set. Spots were manually detected and automatically matched to a master gel selected by the PDQuest software. The spot detection, spot boundary tool and matching were manually edited. The spots were checked and manually added to the master gel to allow matching of unique spots present in the individual gels. Spot quantities were normalized to remove variations non-related to expression changes in spot intensity. The criterion of a differential expression of any particular protein between the experimental groups was set as at least a 2-fold

change in spot volume between matched sets in triplicates. All these spots were selected for subsequent analysis.

### 2.6. Tandem mass spectrometry (LC/ESI-MS/MS)

The identification of the proteins corresponding to the selected spots was performed as described (Fonseca-Sánchez et al., 2012). Briefly, protein spots were excised from Sypro-Ruby stained gels, destained and digested by 20 ng/μl porcine trypsin solution (Promega, Madison, WI, USA) in 50 mM ammonium bicarbonate. Peptides were then analyzed through a 3200 Q TRAP hybrid tandem mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada), equipped with a nano electrospray ion source (NanoSpray II) and a MicroIonSpray II head, using operation conditions previously described (González-Zamorano et al., 2009). Data interpretation and protein identification were performed with the MS/MS spectra data sets using the MASCOT search algorithm (version 1.6b9, Matrix Science, London, UK available at http://www.matrixscience.com). Searches were conducted using the National Center for Biotechnology Information non-redundant database (NCBInr, http://www.ncbi.nih.gov). A protein 'hit' was accepted as a valid identification when at least two MS/MS spectrum matched at the 95% level of confidence ($p < 0.05$). Ion score is $-10$*Log(P), where P is the probability that the observed match is a random event. The threshold ion score in the above conditions was 41 for $p < 0.05$.

### 2.7. Western blot assays

Protein extracts (20 μg) corresponding to *E. histolytica* trophozoites grown in the presence or absence of incomptine A were resolved by 10% SDS–PAGE and electrotransferred to nitrocellulose membranes in wet conditions (Wet electroblotting systems, Bio-Rad). Membranes were blocked with 5% non fat milk in PBS-Tween 0.05% for 2 h at 37 °C. The detection of selected proteins was performed with the next primary antibodies: rabbit polyclonal antibody anti-*E. histolytica* PFO (1:500 dilution) (kindly supplied by Dr. Mario Alberto Rodríguez), and rabbit polyclonal antibody anti-human NUBP2 (1:100) Santa Cruz (sc-135667). The rabbit polyclonal antibody raised against the *E. histolytica* housekeeping lectin was used as control (kindly supplied by Dr. Patricia Talamás). Membranes were incubated with primary antibodies overnight at 4 °C. Corresponding goat anti-mouse or anti-rabbit antibodies coupled with HRP (1:1000, 1 h at 37 °C) were used as secondary antibodies. Proteins were developed by the chemiluminescence method using the Immun-Star WesternC Kit (Bio-Rad) and bands were submitted to densitometric analysis. Lectin data were used to normalize PFO and NUBP2 data in each condition.

### 2.8. Transmission electron microscopy

*E. histolytica* trophozoites were grown with or without incomptine A for 48 h at 37 °C. After that, trophozoites were harvested by chilling at 4 °C, fixed with 3% glutaraldehyde for 2 h and washed three times with a solution containing 0.1 M glutaraldehyde and 1% $CaCl_2$. Cells were post-fixed with 1% osmium tetroxide for 2 h. Trophozoites were dehydrated with increasing concentrations of ethanol (from 10% to 90%) for 10 min, followed by treatment with propylene oxide: alcohol mixtures (2:1, 1:1, and 1:2) for 15 min. Pre-inclusion was done using propylene oxide: resin (2:1, 1:1, and 1:2) for 15 min. Polymerization were done by incubation at 60 °C for 24 h. Thin sections of 70–500 nm were cut and mounted on copper grids. Sections were stained with uranyl acetate for 15 min and lead citrate for 20 min (both from SP1 Supplies; SP1-Chem) and observed through a JEOL-10-10 transmission electron microscope. In this study 40–50 fields of each preparation were analyzed. Ultrastructural changes were quantified using the software Nis Elements from Nikon[MR].

### 2.9. Statistical analyses

Down- and up-regulated spots were analyzed with quantity-test for 2.0-fold and with the Student's *t*-test with intervals of 95%.

## 3. Results

### 3.1. Protein expression is affected by incomptine A treatment

To identify the effect of incomptine A on global protein expression in *E. histolytica* trophozoites, we performed 2-D DIGE assays. Since proteomic profiles corresponding to both control groups were almost similar (data not shown), trophozoites growing in standard medium was used as control group in the following experiments. Fig. 1A shows two representative gels from untreated (left) and incomptine A treated trophozoites (right). The proteomic profiles obtained were highly reproducible in the different experiments. Comparative densitometric analysis of 12 selected spots from untreated and incomptine A-treated trophozoites evidenced the differential protein expression between both proteomic profiles (Fig. 1B).

The comparative analysis of 2-D gels allowed the detection of 21 differentially expressed proteins (>2.0-fold change). Among these proteins, 13 were up-regulated and eight were down-regulated. After LC/ESI-MS/MS tandem mass spectrometry analysis and NCBI database search, 12 modulated proteins were identified: five correspond to down-regulated proteins and seven to up-regulated proteins. The identity and function of proteins, accession number, sequence coverage, Mascot score, and MS/MS peptide sequence (ion score) are summarized in Tables 1 and 2. Interestingly, down-regulated proteins included three enzymes of the glycolytic pathway, namely enolase, pyruvate: ferredoxin oxidoreductase (PFO) and fructose-1,6-biphosphate aldolase. Other down-regulated proteins were a membrane transporter protein (ATP binding cassette protein), and an oxidative stress-associated protein (thioredoxin reductase). On the other side, up-regulated proteins included a protein related with protein synthesis (eIF5A), an iron homeostasis and centrosome duplication protein (NUBP2), a cytoskeleton component protein (cortexillin), a vesicular traffic-related protein (a soluble NSF attachment protein) and two hypothetical proteins. Proteomic results were validated by western blot assay using antibodies raised against one under-expressed and one over-expressed protein after incomptine A treatment: PFO and NUBP2, respectively (Fig. 2). Results evidenced a weaker expression of PFO (3%), while NUB2 was over-expressed (123%) in response to incomptine A effect (Fig. 2C), which was in agreement with the differential expression detected in proteomic profiles.

### 3.2. Incomptine A treatment induces ultrastructural changes in E. histolytica trophozoites

Since proteomics data suggested that incomptine A has an inhibitory effect on enzymes of the glycolytic pathway in *E. histolytica*, trophozoites ultrastructure was analyzed through transmission electronic microscopy in order to evidence changes related with glycolysis failure. The majority of the trophozoites (93%) treated with incomptine A exhibited important ultrastructural changes in comparison to untreated cells (Fig. 3). Remarkably, we observed an 88% increase in the number of glycogen granules, a 47% reduction in the number of vacuoles and a 44.4% reduction in vacuolar area, in the presence of incomptine A (Fig. 4). In addition, 10% of treated trophozoites showed chromatin rearrangements and 17%

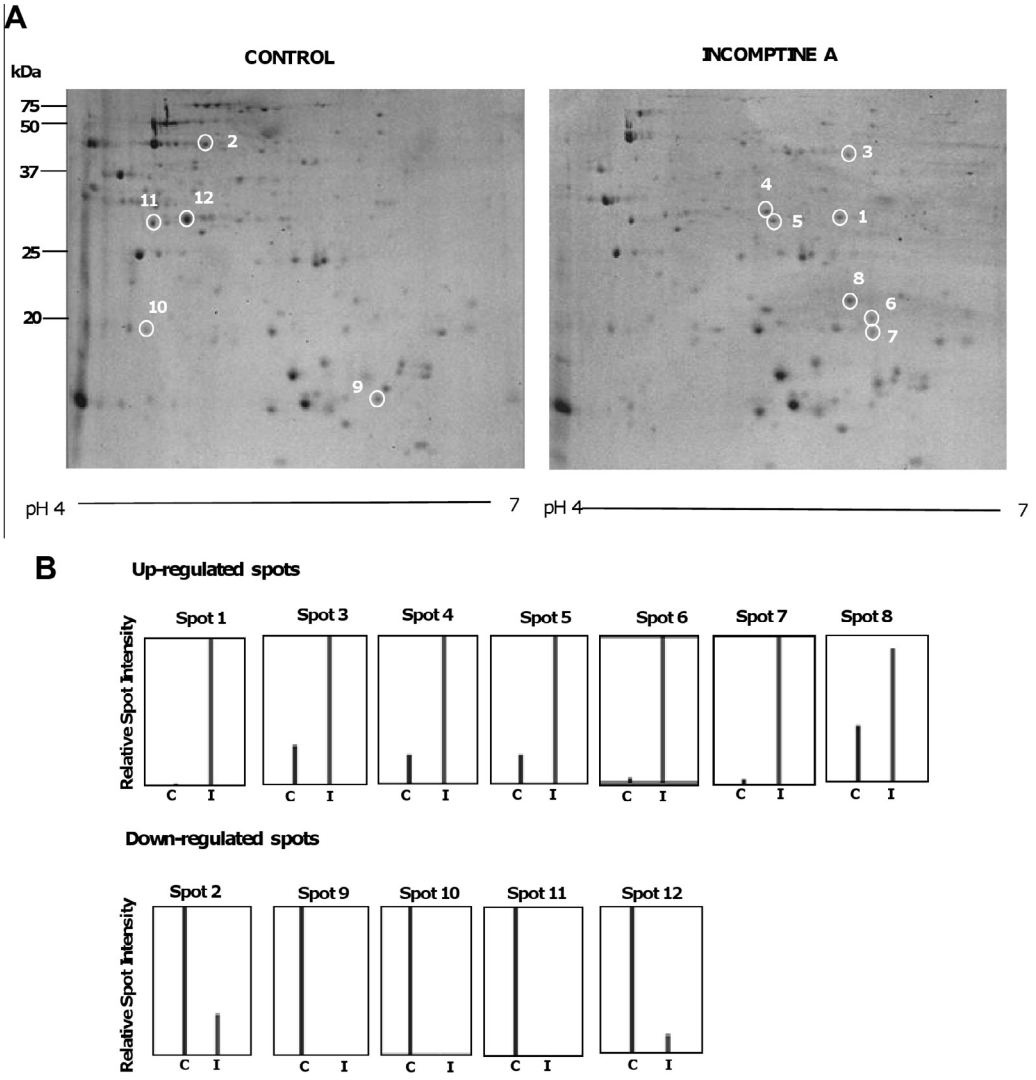


**Fig. 1.** Proteomic analysis of *E. histolytica* trophozoites in response to incomptina A treatment. (A) Representative 2-D DIGE gel (pH range of 4.0–7.0) of *E. histolytica* trophozoites untreated (left) and treated with incomptine A (right). Open circles show differentially expressed protein spots. (B) Densitometric analysis of selected protein spots. The histograms show relative protein spot quantities. Spots were determined as significantly differentially expressed according to Student's *t*-test ($p < 0.05$).

**Table 1**
Down-regulated proteins in *E. histolytica* trophozoites treated with incomptine A.

| Protein (Spot Num) | Accession number[a] | Mascot score | No. of matched peptides | Molecular mass/pI | Sequence coverage (%) | MS/MS peptide sequence (ion scores) | Function |
|---|---|---|---|---|---|---|---|
| Enolase (2) | P51555 | 610 | 23 | 47924/5.87 | 49 | $^{16}$GNPTIEVEITTGK$^{28}$ (83)<br>$^{33}$SCVPSGASTGVHEAVELR$^{50}$ (102)<br>$^{65}$AVENVNTIIGPALLGK$^{80}$ (82)<br>$^{81}$NVLNQAELDEMMIK$^{94}$ (72) | Glycolysis, gluconeogenesis, plasminogen receptor |
| ATP-binding cassette protein (9) | B1N2M5 | 49 | 2 | 155522/6.07 | 2 | $^{2}$KEEQK$^{9}$ (29)<br>$^{3}$KQENESSDSTEKPTELIDLNTPSASPMPLDGTVTNKD$^{38}$ (22) | Transport, gene translation and regulation, DNA repair, drug resistance |
| Thioredoxin reductase (11) | C4LW95 | 678 | 16 | 34062/5.93 | 61 | $^{2}$SNIHDVVIIGSTGPAAHAAIYLGR$^{25}$ (76)<br>$^{2}$MSNIHDVVIIGSGPAAHTAAIYLGRS$^{25}$ (73) | Oxidative stress prevention |
| Fructose-1,6-bisphosphate aldolase (12) | C4LXD7 | 979 | 65 | 36479/6.07 | 66 | $^{10}$ELGLCNHK$^{17}$ (11)<br>$^{18}$EMFEHAIK$^{25}$ (27)<br>$^{26}$GGFAVPGFNFNNLEQMQAIIQACTEAK$^{52}$ (93)<br>$^{65}$EYANATLLR$^{73}$ (56)<br>$^{110}$ECVDNGFSNVMIDGSALPYEENVK$^{133}$ (91) | Glycolytic pathway, gluconeogenesis |
| Pyruvate ferredoxin oxidoreductase (10) | C4LXD7 | 198 | 10 | 129794/6.62 | 4 | $^{678}$IPTWEASK$^{685}$ (51)<br>$^{756}$CLSMTPFEQVSEVESK$^{771}$ (93) | Glycolytic pathway |

[a] UniProt Knowledge Database.

**Table 2**
Up-regulated proteins in *E. histolytica* trophozoites treated with incomptine A.

| Protein (Spot Num) | Accession number[a] | Mascot score | No. of matched peptides | Molecular mass/pI | Sequence coverage (%) | MS/MS peptide sequence (ion scores) | Function |
|---|---|---|---|---|---|---|---|
| Nucleotide binding protein 2 (1) | C4LZB2 | 341 | 16 | 29611/4.98 | 61 | $^{9}$NFVGVDHVK$^{18}$ (32) $^{18}$NVILVLSGK$^{26}$ (39) | Iron homeostasis, apoptosis, centrosome duplication |
| Cortexillin (3) | C4M741 | 738 | 22 | 89552/5.07 | 22 | $^{410}$NLELEQEQK$^{417}$ (32) | F actin crosslinking protein, cytokinesis |
| Hypothetical protein (4) | C4M2U3 | 325 | 11 | 27836/5.41 | 41 | $^{13}$AIFIETVTNFAQK$^{25}$ (74) $^{33}$VFLQILPLTVVAGTEYLK$^{50}$ (77) | DNA degradation and repair of the DNA, cellular cycle |
| Alpha-soluble NSF attachment protein (5) | C4M0D4 | 648 | 14 | 32441/5.34 | 50 | $^{30}$EANMTDAAELFNK$^{42}$ (94) | Repair proteins on damaged chromosomes, ubiquitination and proteosomal degradation, membrane fusion machinery, epithelial junctions |
| Translation initiation factor eIF-5A (6) | C4LVN8 | 286 | 12 | 17349/5.13 | 50 | $^{30}$KGGYVVIK$^{37}$ (39) $^{59}$ VTFAGYDIFTGK$^{70}$ (96) $^{72}$LDDSCPSTHNVEVPEVK$^{88}$ (46) | Synthesis of proteins, Apoptosis (p53), translation of specific mRNAs, cell proliferation and differentiation |
| Translation initiation factor eIF-5A (7) | C4LVN8 | 151 | 4 | 17349/5.13 | 3 | $^{8}$KGGYVVIKG$^{16}$ (17) $^{77}$KVTFAGYDIFTGK$^{88}$ (89) | Synthesis of proteins, apoptosis, translation of specific mRNAs, cell proliferation and differentiation |
| Hypothetical protein (8) | C4M2S1 | 106 | 6 | 21439/4.95 | 36 | $^{24}$KFLIVFK$^{30}$ (30) $^{25}$FLIVFK$^{30}$(33) $^{62}$QVTSALQSCGEPK$^{74}$ (45) | Putative uncharacterized protein |

[a] UniProt Knowledge Database.

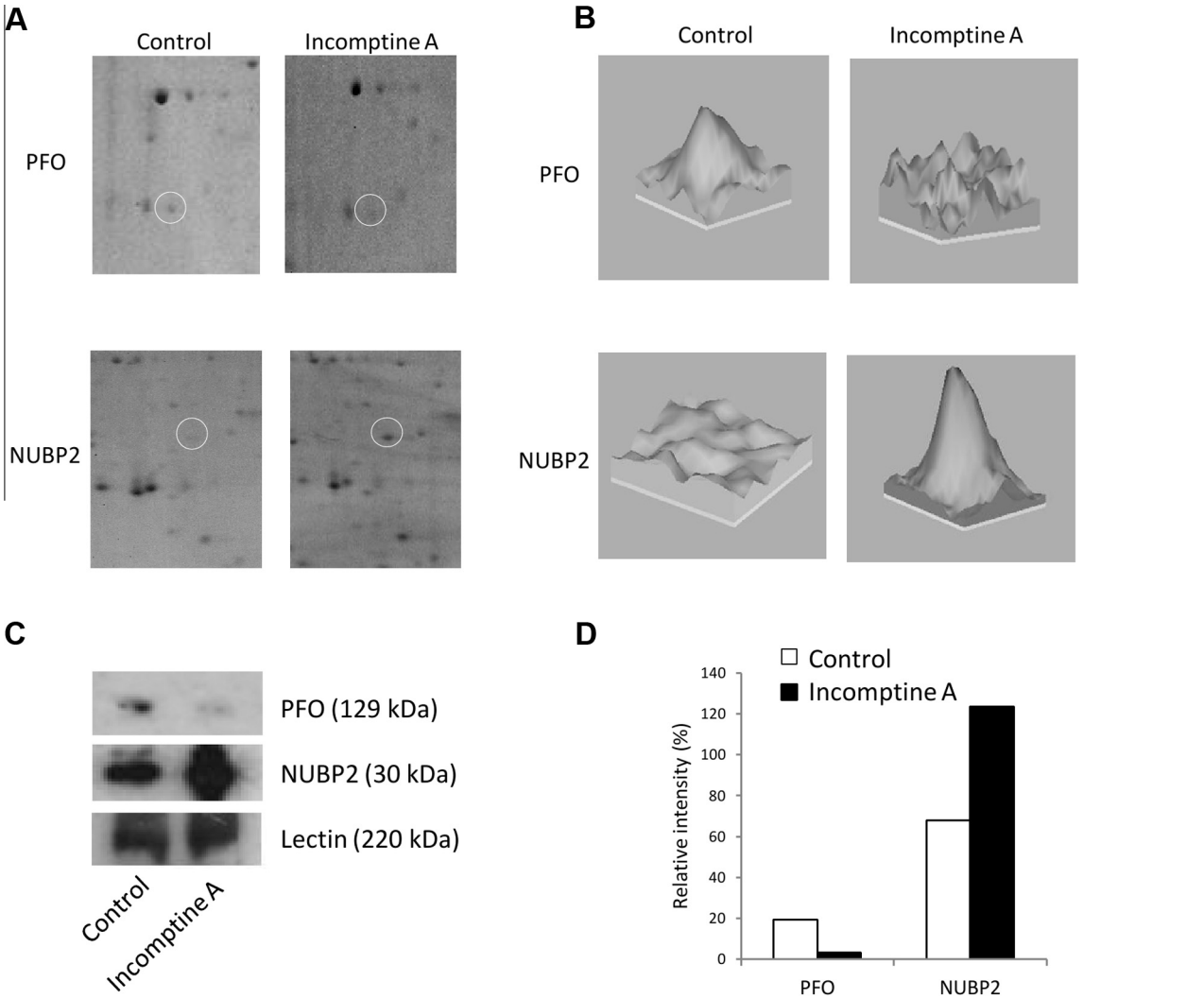


**Fig. 2.** Expression of PFO and NUBP2 in *E. histolytica* trophozoites in response to incomptine A treatment. (A) Cropped representative 2-D gel images of PFO and NUBP2 spots (denoted as open circles) in incomptine A treated (right) and untreated (left) trophozoites. (B) Volume of the PFO and NUBP2 spots were calculated using Melanie software and are graphically represented. (C) PFO and NUBP2 expression analyzed by western blot assay using anti-PFO and anti-NUBP2 polyclonal antibodies. Lectin was used as an internal loading control. (D) Densitometric quantification of PFO and NUBP2 bands shown in (C). Lectin was considered as 100% in each condition.

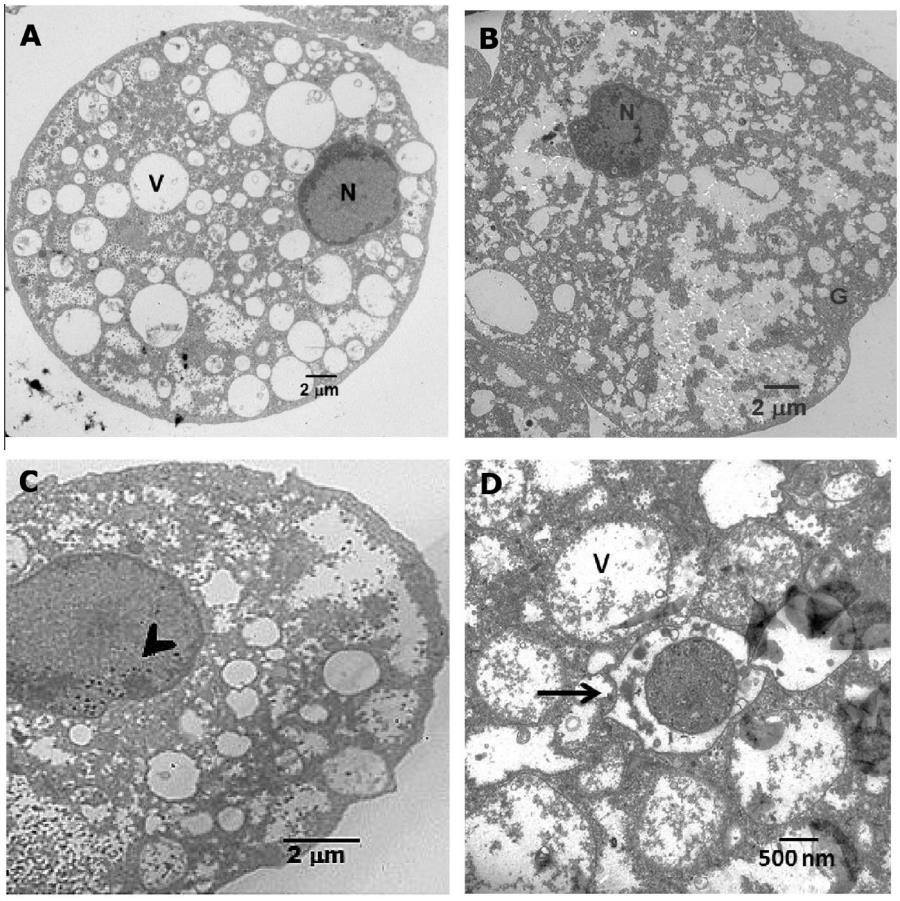


**Fig. 3.** Transmission electron microscopy of trophozoites without treatment (A) and treated with incomptine A (B–D). N, nucleus; V, vacuole; G, glycogen deposit. Arrowhead indicates chromatin redistribution; arrow is pointing to membranous structures inside vacuoles. The scale (bar) is indicated in each panel.

contained membranous structures inside vacuoles, resembling autophagosomes. Incomptine A did not induce any alterations in cellular and nuclear membranes or significant change in cell size.

## 4. Discussion

The STL incomptine A was discovered from *D. incompta* leaves in an attempt to develop new drugs that can help to the control of amoebiasis, which remains a highly prevalent intestinal disease in most developing countries. A previous study showed that its antiprotozoal activity could be due to the diene and acetate group in C-8 of the germanacrane rings (Bautista et al., 2012) but the molecular mechanisms responsible for the antiparasitic activity of this compound are still unknown.

Here, we performed a traditional two-dimensional gel electrophoresis in order to contribute to the identification of *E. histolytica* proteins whose expression is affected by incomptine A. The comparative analysis of proteomic profiles evidenced that incomptine A induced the down-regulation of three glycolytic enzymes, namely enolase, PFO and fructose-1,6-biphosphate aldolase, suggesting that this compound could be inducing a reduction of glucose metabolism in trophozoites. This hypothesis was supported by the ultrastructural alterations observed in incomptine A-treated trophozoites, such as the increase in cytoplasmic glycogen granules. This alteration has been previously reported in G. *lamblia* trophozoites grown in the presence the alkaloid berberine sulphate and this finding was associated with the inhibition of the parasite glycogen metabolism (Kaneda, 1991). Since *E. histolytica* trophozoites rely exclusively on glycolysis for ATP supply, as they are devoid of Krebs cycle and oxidative phosphorylation enzymes (Reeves, 1984; McLaughlin and Aley, 1985), the inhibition of parasite glycolytic enzymes could explain the antiprotozoal activity of incomptine A.

The effect of other STL on glycolytic metabolism has been previously reported in mammalian cells. Notably, STL induced a decrease in glucose consumption and lactic acid formation, as well as an inhibition of different enzymes, including phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase, pyruvate kinase, lactate dehydrogenase, hexokinase, (Duszenko et al., 1982; Baer et al., 1983; Gaspar et al., 1986), glycogen synthetase, aldolase, phosphorylase, glucose-6-phosphatase, and succinic dehydrogenase. Enzymatic inhibition appears to be by alkylation of functional groups, *e.g.*, sulfhydryl groups of these enzymes, by a rapid Michael-type addition with the alpha-methylene-gamma-lactone moiety of sesquiterpene lactones (Hall et al., 1978, 1980). The alkylation of amino acid containing sulfhydryl group (mostly cysteine), could also be affecting the proper three dimensional structure of the enzyme, the intrinsic charge, the isoelectric point, as it has been reported previously during the alkylation of other proteins (Bouchon et al., 2005). Here we did not evaluate the activity of parasite glycolytic enzymes. However, it is possible that incomptine A induced similar alkylation reactions in *E. histolytica* enzymes which could explain the reduction of the spots corresponding to PFO, enolase, and fructose-1,6-biphosphate aldolase. Another hypothesis is that incomptine A could inhibit glycolytic enzymes expression by interfering with some regulatory proteins as it has been suggested for other STL. García-Piñeres et al., 2001,

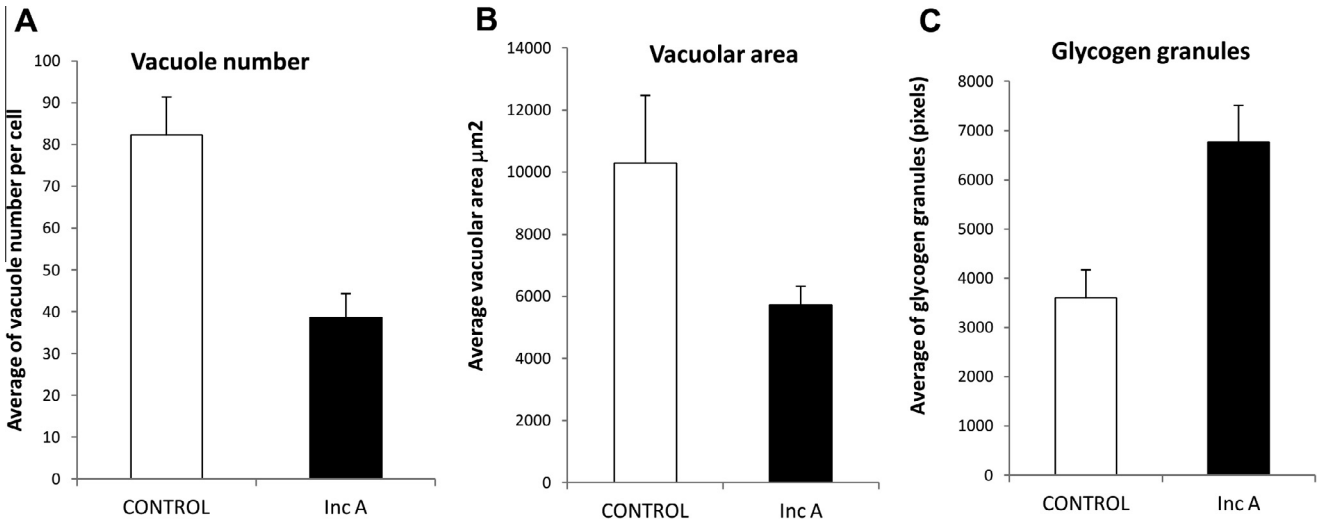


**Fig. 4.** Effects of incomptine A on *E. histolytica* trophozoite ultrastructure. The number of vacuoles per trophozoite (A), the vacuolar area per cell (B), and the number of glycogen granules per trophozoites (C), were determined in control trophozoites (without treatment) and incomptine A treated trophozoites (Inc A) using the software Nis Elements from Nikon.

reported that the STL parthenolide exerts its anti-inflammatory properties by inhibiting directly the transcription factor NF-kB by alkylating cysteine 38 sulfhydryl groups in its p65 subunit. Thus, NF-kB can not bind to promoters of NF-kB dependent genes, avoiding their transcription. Other studies indicated that parthenolide inhibits I-kK (the kinase finally phosphorylating IkB α) and/or reacts directly with IkB α (NF-kB inhibitor) preventing its phosphorylation (Hehner et al., 1998, 1999; Kwok et al., 2001; Lopez-Franco et al., 2002). Additionally, several reports suggest that parthenolide can inhibit p38 MAPK, JNK, and other kinases and may stabilize some phosphorylated intermediates (Hwang et al., 1996; Uchi et al., 2002). Further studies are required to determine the exact molecular mechanisms by which the STL incomptine A affects *E. histolytica* glycolytic pathway and exerts its antiparasitic effects.

Enzymes that only exist in parasites and not in human host, have always been considered as attractive biochemical targets for the development of new drugs. One relevant difference between glucose metabolism of *E. histolytica* and human is the presence of a metal-dependent clase II fructose-1,6-bisphosphate aldolase in trophozoites, which has no homologues in human cells (Saavedra et al., 2005). Moreover, pyruvate is converted to acetyl-CoA by PFO instead of a pyruvate dehydrogenase complex (Saavedra-Lira and Pérez-Montfort, 1996). Additionally, protein modeling studies showed that *E. histolytica* enolase possesses a specific region that differs from human enolase (Hidalgo et al., 1997). Differences found between parasite and host glycolytic enzymes could help to design a specific inhibitor of *E. histolytica* glycolytic pathway using incomptine A as a leader molecule.

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