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# Transcriptional profiling of *Entamoeba histolytica* trophozoites

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**Abstract**

We have developed an *Entamoeba histolytica* genomic DNA microarray and used it to develop a transcriptional profile of 1,971 *E. histolytica* (HM-1:IMSS) genes. The arrays accurately detected message abundance and 31–47% of amebic genes were expressed under standard tissue culture conditions (levels detectable by Northern blot analysis or RT-PCR respectively). Genes expressed at high levels (~2% of total) included actin (8.m00351), and ribosomal genes (20.m00312). Moderately expressed genes (~14% of total) included cysteine proteinase (191.m00117), profilin (156.m00098), and an Argonaute family member (11.m00378). Genes with low-level expression (~15% of total) included *Ariel*1 (160.m00087). Genes with very low expression (~16% of total) and those not expressed (~52% of total) included encystation-specific genes such as Jacob cyst wall glycoprotein (33.m00261), chitin synthase (3.m00544), and chitinase (22.m00311). Transcriptional modulation could be detected using the arrays with 17% of genes upregulated at least two-fold in response to heat shock. These included heat shock proteins (119.m00119 and 279.m00091), cyst wall glycoprotein Jacob (33.m00261), and ubiquitin-associated proteins (16.m00343; 195.m00092). Using Caco-2 cells to model the host-parasite interaction, we verified that host cell killing was dependent on live ameba. However, surprisingly these events did not appear to induce major transcriptional changes in the parasites.


*Keywords:* Ameba; Expression; DNA microarrays; Host-parasite interaction

## 1. Introduction

The protozoan parasite *Entamoeba histolytica* causes an estimated 50 million cases of invasive disease and approximately 100,000 deaths each year (World Health Organization, 1997). The most common manifestations of amebic infection are dysentery and liver abscesses (Haque et al., 2003). Infection begins with ingestion of cysts, excystation of cysts into trophozoites, and migration of the trophozoites to the colon where they either asymptomatically colonize, cause invasive disease, or reencyst. The molecular mechanisms that regulate these events are not well characterized.

A number of genes of *E. histolytica* have been implicated in amebic colonic pathogenesis (Espinosa-Cantellano and Martinez-Palomo, 2000; Stanley, 2003). Among these are the Gal/Gal-NAc lectin, the primary factor responsible for attachment to and killing of host cells (Petri, 1996). This complex also has critical roles in complement resistance, induction of apoptosis, and inside out signaling (Braga et al., 1992; Vines et al., 1998; Huston et al., 2000, 2003). Cysteine proteases, a family of cathepsin proteases are largely responsible for degrading mucin and the extracellular matrix of the colonic epithelium (Que and Reed, 2000). These proteases also mediate complement resistance (by lysis of the C3a and C5a complexes), inactivate interleukin-18, and have interleukin-1 beta converting enzyme activity (Reed et al., 1989; Zhang et al., 2000; Que et al., 2003). Amoebapores, pore-forming peptides, are involved in lysis of target cells and ingested bacteria (Leippe et al., 1994; Leippe, 1995). A number of other amebic derived proteins (glycosidases

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and collagenases) are important in causing invasive colonic disease (Espinosa-Cantellano and Martinez-Palomo, 2000).

Genetic proof of the roles of some genes in invasive disease has been obtained. Antisense inhibition of cysteine proteinases result in trophozoites with reduced phagocytic capacity in vitro, and decreased colonic epithelial inflammation/tissue injury and liver abscess formation in animal models (Ankri et al., 1998, 1999a,b; Zhang et al., 2000). Antisense inhibition of the light subunit of the lectin results in reduced cytolytic capacity and inability to form liver abscesses in animal models (Ankri et al., 1999a,b). Similar effects on cytolytic activity are also seen when amebapore expression is reduced by antisense inhibition (Bracha et al., 1999). However, recently it has been shown that *E. histolytica* silenced for amebapore A, although abrogated in its ability to cause liver abscesses, is still able to cause disease in a mouse model of colitis, implying that amebapores play lesser roles in colonic disease (Zhang et al., 2004). Phagocytosis and motility are also linked to virulence and parasites genetically manipulated to be deficient in these parameters are attenuated in a number of virulence assays (Rodriguez and Orozco, 1986).

*E. histolytica* regulates its transcriptional profile under a number of conditions including drug resistance, oxidative stress, nutrient deprivation, and collagen-calcium exposure (Samuelson et al., 1990; Sanchez et al., 1994; Park et al., 2001; Akbar et al., 2004; Debnath et al., 2004). Parasites from hepatic infections have a novel transcriptional profile as compared with trophozoites in tissue culture (Bruchhaus et al., 2002). Caco-2 cells have been used as a model to study ameba-enteric cell interactions, however, relatively little is known about the transcriptional response of parasites after the parasites contact the enteric target cell (Rigothier et al., 1991; Li et al., 1994). Studies have demonstrated that ameba upon attaching to polarized Caco-2 cells, decrease transepithelial resistance, increase epithelial permeability, and disrupt the apical brush border architecture (Li et al., 1994). Quantitative measurements of virulence determinants have not been made after enterocyte interaction, although it has been demonstrated that the amebic lectin is transferred to the host cell surface after contact (Leroy et al., 1995). Trophozoites of *Entamoeba* have also been shown to transfer lipophosphopeptidoglycans to the apical side of enterocytes and parasite cysteine proteinases cause proteolysis of villin on the enteric cell surface (Lauwaet et al., 2003, 2004).

Determining the molecular basis for the initial invasion step is critical to understanding the pathogenesis of *E. histolytica*. Using a genomic DNA microarray we have characterized the expression profile of 1,971 amebic genes when *E. histolytica* trophozoites interact with enteric host cells. This first large-scale analysis of amebic transcriptional patterns unexpectedly revealed that the host cell interaction does not significantly modulate the amebic transcriptional profile.

## 2. Materials and methods

### 2.1. Microarray generation

A genomic DNA microarray was generated for *E. histolytica* (HM-1:IMSS) as described previously (Shah et al., 2005). Briefly, sequenced clones (from the random sheared genomic library used for genome sequencing) were PCR-amplified, cleaned, and robotically spotted onto poly-L-lysine coated glass slides. Two arrays were generated in this manner using identical reagents and techniques. A smaller array with 1,500 clones (containing 360 unique genes) was used for heat shock experiments to validate utility of the arrays. A second larger array with 11,328 clones (containing 2,112 unique genes) was used for expression profiling and host-parasite interaction studies. Correct clone assignment was ascertained and clones that cross-hybridized with host RNA were removed from analysis. Information from genome annotation was used to identify clones that contained only one potential open reading frame (ORF) (defined as $\geq$98% identity over $\geq$200 bp).

### 2.2. Parasite and Caco-2 cell culture

*E. histolytica* (HM-1:IMSS) were grown under axenic conditions in trypticase-yeast extract-iron-serum medium (TYI-S-33) with 15% adult bovine serum (Sigma), supplemented with penicillin (100 U/ml), and streptomycin (100 μg/ml) (Gibco BRL) at 37 °C as previously described (Diamond et al., 1978). Caco-2 cells (kindly provided by Dr James Nelson, Stanford University) were grown in Dulbecco's minimal essential media supplemented with 10% bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) as previously described (Lauwaet et al., 2003). Cells were allowed to polarize by growing for a minimum of 10 days post confluency at which time characteristic features of polarization were identified. For heat shock experiments, log phase *E. histolytica* (HM-1:IMSS) trophozoites were subjected to 42 °C for 1 h, harvested, and RNA isolated by methods outlined below. A parallel culture was maintained at 37 °C and used as an unactivated control.

### 2.3. Ameba:Caco-2 cell interaction

Log phase *E. histolytica* HM-I:IMSS were applied to polarized Caco-2 cells at a ratio of 1 ameba:15 Caco-2 cells in TYI-S-33 media. At 1, 3, 6, 9 h after host-parasite interaction, the media was removed and the monolayer rinsed with warm PBS. Ice cold PBS with 100 mM galactose were added for 5–10 min to remove adherent ameba from the host cell monolayer (Huston et al., 2003). Parallel amebic cultures grown simultaneously in the absence of Caco-2 cells, but otherwise treated identically to those described above were used for ameba alone control arrays. Caco-2 monolayer destruction was monitored by