Journal of Microbiology, Immunology and Infection (2015) 48, 662–675

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Gene-expression analysis of cold-stress response in the sexually transmitted protist *Trichomonas vaginalis*

Yi-Kai Fang [a,b], Kuo-Yang Huang [b], Po-Jung Huang [c], Rose Lin [b], Mei Chao [a,d], Petrus Tang [a,b,c,*,d]

[a] *Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[b] *Molecular Regulation and Bioinformatics Laboratory, Department of Parasitology, College of Medicine, Chang Gung University, Taoyuan, Taiwan*
[c] *Bioinformatics Center, Chang Gung University, Taoyuan, Taiwan*

Received 17 July 2014; accepted 7 August 2014
Available online 1 November 2014

KEYWORDS
Cold stress;
Expressed sequence tag;
Trichomonas vaginalis

Abstract *Background: Trichomonas vaginalis* is the etiologic agent of trichomoniasis, the most common nonviral sexually transmitted disease in the world. This infection affects millions of individuals worldwide annually. Although direct sexual contact is the most common mode of transmission, increasing evidence indicates that *T. vaginalis* can survive in the external environment and can be transmitted by contaminated utensils. We found that the growth of *T. vaginalis* under cold conditions is greatly inhibited, but recovers after placing these stressed cells at the normal cultivation temperature of 37°C. However, the mechanisms by which *T. vaginalis* regulates this adaptive process are unclear.

Methods: An expressed sequence tag (EST) database generated from a complementary DNA library of *T. vaginalis* messenger RNAs expressed under cold-culture conditions (4°C, TvC) was compared with a previously published normal-cultured EST library (37°C, TvE) to assess the cold-stress responses of *T. vaginalis*.

Results: A total of 9780 clones were sequenced from the TvC library and were mapped to 2934 genes in the *T. vaginalis* genome. A total of 1254 genes were expressed in both the TvE and TvC libraries, and 1680 genes were only found in the TvC library. A functional analysis showed that cold temperature has effects on many cellular mechanisms, including increased H_2O_2

* Corresponding author. Molecular Regulation and Bioinformatics Laboratory, Department of Parasitology, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Taoyuan 333, Taiwan.
E-mail address: petang@mail.cgu.edu.tw (P. Tang).
[d] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jmii.2014.07.013

tolerance, activation of the ubiquitin—proteasome system, induction of iron—sulfur cluster assembly, and reduced energy metabolism and enzyme expression.
Conclusion: The current study is the first large-scale transcriptomic analysis in cold-stressed *T. vaginalis* and the results enhance our understanding of this important protist.

Introduction

Trichomoniasis, caused by the protozoan *Trichomonas vaginalis*, is the most common nonviral sexually transmitted disease (STD) in the world, and affects millions of people worldwide annually.[1] This infection is usually asymptomatic in men,[2] but symptoms in women can include vaginal discharge, pruritus, odor, irritation, and edema or erythema.[3] Trichomoniasis is also associated with preterm delivery in pregnant women, low infant birth weights,[3] human immunodeficiency virus transmission,[4,5] cervical cancer,[6] and prostate cancer.[7] The clinical treatment of trichomoniasis includes administration of metronidazole, a 5-nitroimidazole compound, which effectively eliminates *T. vaginalis* infection,[8] although increasing clinical cases of drug-resistance isolates have demonstrated the need for new drug development.[9]

It has long been known that *T. vaginalis* can be transmitted directly between hosts by sexual contact. However, cumulated evidence has shown that *T. vaginalis* may also cause infections in people who have not engaged in sexual activity, which implies a nonsexual route of *T. vaginalis* transmission.[10,11] For transmission through contaminated utensils to be possible, *T. vaginalis* must have adaptive mechanisms to the external environment. It was reported that this protist has a strong cold tolerance and that it can survive in culture medium for over 1 week at 4°C in comparison with 2 days at normal-cultured temperature, 37°C.[12] Thermal stress is common to many cell types, and it often triggers the expression of various genes and adaptive responses. The principal cellular mechanisms of the cold-stress response are reductions in the enzymatic reaction rate, diffusion, and membrane transport, as well as the inhibition of transcription and translation, increased protein denaturation and aggregation, and the disruption of cytoskeletal elements.[13]

Currently, there are studies on the cold-adaptive response of *T. vaginalis*. To gain insights into the gene-expression reprogramming during cold-stressed conditions, we performed large-scale genomic assays to identify expressed sequence tags (ESTs). The complementary DNA (cDNA) library (TvC) was constructed from *T. vaginalis* messenger RNAs (mRNAs) expressed during cultures at cold temperature (4°C). This gene-expression profile was compared with a normal-cultured *T. vaginalis* EST data set (TvE) published previously[14] to assess the changes in gene expression under cold-stressed conditions and thereby analyze the cold-stress response of *T. vaginalis*.

Methods

Cell culture and counting

T. vaginalis C-1:NIH (ATCC strain 30001) was maintained axenically at 37°C in YI-S medium (pH 5.8) supplemented with 10% heat-inactivated horse serum.[15] For the cold treatment, logarithmic-phase protists cultured at 37°C were transferred to a 4°C culture for 4 hours. To determine the proliferation ability of cold-stressed *T. vaginalis*, approximately 2×10^5 cells/mL of normal-cultured protists were placed in a 25°C or 4°C environment for different periods and then returned to a 37°C environment for another 12 hours to recover. The number of viable cells before and after the 37°C recovery was counted by trypan blue exclusion with hemocytometer.

cDNA library construction and ESTs sequencing

The RNA extraction, cDNA library construction, and the sequencing of the tags were performed as previously described.[14] All sequences are available on European Nucleotide Archive (http://www.ebi.ac.uk/ena/) with accession numbers from LK985528 to LK995307.

Reverse transcription and quantitative polymerase chain reaction

Total RNA was extracted from normal and cold-treated *T. vaginalis* by TRIzol reagent as described earlier. Five micrograms of extracted RNA was then used for reverse transcription using SuperScript III first-strand synthesis system (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions. The quantitative polymerase chain reaction (qPCR) was carried out as previously described,[16] in which 50 ng of reverse transcription product was mixed with Master Mix (Ampliqon A/S, Odense, Denmark) and 0.5μM forward and reverse primers. These primer sequences are listed in Table 1.

Proteasome activity assay

Analysis of proteasome activity was performed using the proteasome activity fluorometric assay kit (BioVision Inc., Milpitas, CA, U.S.A.) according to the manufacturer's instructions. In brief, 2×10^5 cells from normal and cold-stressed cultures were lysed in 0.5% NP-40 and loaded into a 96-well plate in paired wells. One microliter of MG-