Transcriptome analysis of *Nageleria fowleri* cysts and trophozoites by Next-Generation Sequencing

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ABSTRACT

***Naegleria fowleri*,** ubiquitous pathogenic amoeba causing the fatal primary amoebic meningoencephalitis (PAM) in experimental animal and humans, is predominantly living in the ponds, lakes, rivers and swimming pools. *N. fowleri* trophozoites are encysted under unfavorable conditions such as cold temperature, starvation and desiccation. **However, the information in differential expression genes between cysts and trophozoites of *N. fowleri* is very limited. In this study, RNA-sequencing libraries from *N. fowleri* cysts and trophozoites were investigated by Next-Generation Sequencing (NGS) analysis.** In the NGS database, the assembly procedure resulted in mean full length of 11, 254 nucleotides in total 42,220 transcript contigs and 37.21 % of C+G contents. RNA sequencing indicated that upregulated 143 genes in cysts showed 2 folds expression in comparison with trophozoites and 163 genes were downregulated. These genes were found to participate in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. KEGG pathway included metabolisms (131), cellular processes (43), environmental Information processing (22), genetic information processing (66) and organismal systems (20). On the other hands, by analysis of 10,713 sequences via the gene ontology database, their annotations included biological processes (1,069) which were cellular process (228), metabolic process (214) and single organisms process (193), molecular functions (415) containing catalytic activity (195) and binding (186) and cellular components (923) possessing cells (240) and cell parts (225). **Increased differential expression transcriptome levels in *N. fowleri* cysts compared to trophozoites were mainly categorized as serine/threonine protease, kinase, and lipid metabolisms related protein.** Finally, this study may provide new insights into the environmental resistant genes or pathogenic related genes in *N. fowleri* survival and infectivity.

Kewords; *Naegleria fowleri*, cyst, trophozoite, Next-Generation Sequencing, transcriptome

INTRODUCTION

★ ***Naegleria fowleri* (brain eating amoeba)** : Ubiquitous pathogenic free-living amoeba
- Primary amoebic meningoencephalitis (PAM) in laboratory animal and humans
- Infection routes: Nasal cavity → mucosal membrane → nasal nerve → olfactory bulb → meninges → encephalitis (brain inflammation) → death

★ **RNA sequencing** (Next-Generation Sequencing) : powerful tool analyzing gene expression levels, comparing differential gene expression

MATERIALS AND METHODS

A. Cultivation and encystation of *N. fowleri* : *N. fowleri* trophozoites (Carter NF69; ATCC No. 30215) were axenically cultured in Nelson's media at 37℃. To induce encystation, *N. fowleri* trophozoites were transferred into an encystation medium.

B. RNA extraction : After 2-day incubation in the encystation medium(95mM NaCl, 5mM KCl, 8mM $MgSO_4$, 0.4mM $CaCl_2$, 1mM $NaHCO_3$, 20mM Tris-Cl(pH9.0)), the cells were harvested for extraction of the total RNA.

C. Library preparation and sequencing

D. De novo transcriptome assembly and analysis

E. Annotation and quantification of the transcriptome

RESULTS

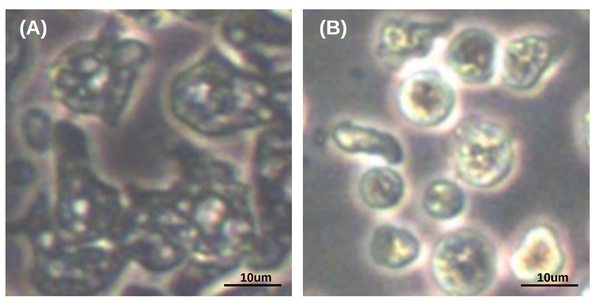
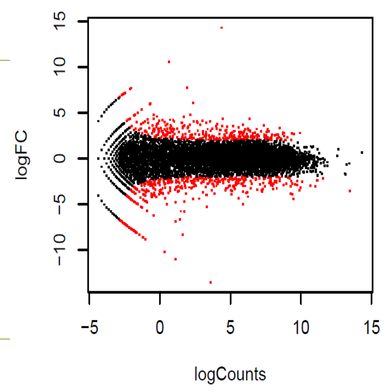


Fig. 1. Live image of *N. folweri* trohpozoites (A) and encystic forms (B) (x200).

Table 1. Characteristics of the transcriptome in *N. fowleri* cysts and trohozoties.

Contig count	42,220
Type	De novo assembly
Total read count (trophozoites and cysts)	135,733,193
Mean read length (nucleotides)	1180.5
Total read length (nucleotides)	33,118,105
Mean contig length (nucleotides)	2471.5
Total contig length (nucleotides)	33,118,105



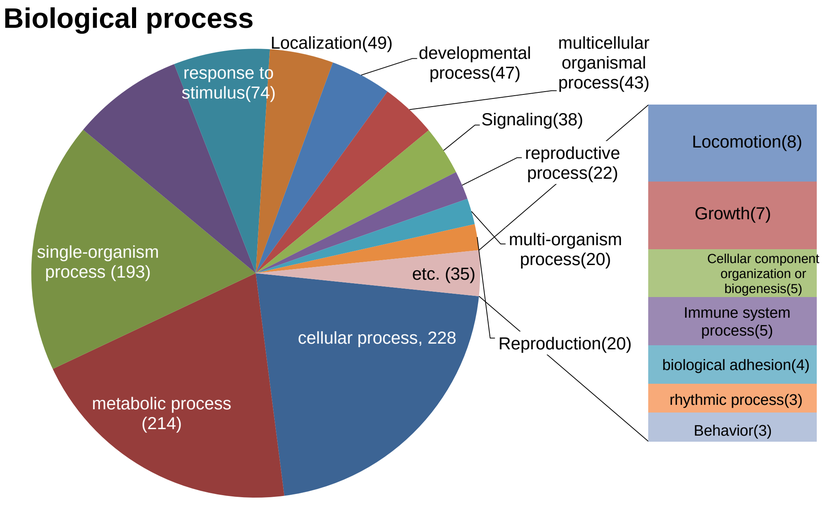
Design	UP DEG (FDR<0.05)	Down DEG (FDR<0.05)	SUM
Control : Trophozoites Case : Cysts	146	163	774

Fig 2. Differential Expression Analysis of the *N. folweri* trohozoties and cysts. The assembly procedure results in 42,220 contigs with a mean length of 11,254 nucleotides and a C+G content of 37.21%. The UP (146) and Down (163) Differential Expression Gene (DEG;●) were analyzed with the Gene Ontology (GO) data base.

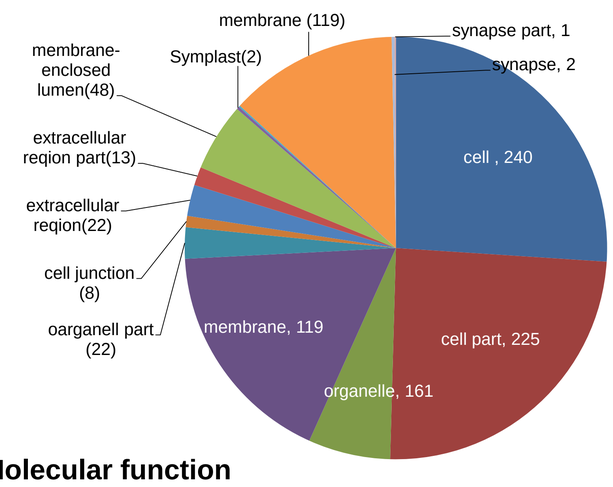
Table 3. Pathway in *N. fowleri* cysts and trophozoites mapped by the Kyoto Encylclopedia Genes and Genomes (KEGG).

Pathway	
Cellular Processes	43
Cell motility	22
Cell growth and death	10
Cellular community	4
Transport and catabolism	7
Environmental Information Processing	22
Signal transduction	12
Membrane transport	7
Signaling molecules and interaction	3
Genetic Information Processing	66
Translation	31
Folding, sorting and degradation	23
Replication and repair	12
Metabolisms	131
Lipid metabolism	22
Energy metabolism	11
Metabolism of other amino acids	9
Nucleotide metabolism	19
Carbohydrate metabolism	37
Amino acid metabolism	8
Metabolism of terpenoids and polyketides	3
Xenobiotics biodegradation and metabolism	6
Metabolism of cofactors and vitamins	8
Glycan biosynthesis and metabolism	2
Biosynthesis of other secondary metabolites	6
Organismal Systems	20
Immune system	5
Digestive system	5
Nervous system	4
Development	1
Endocrine system	5

A. Biological process



B. Cellular component



C. Molecular function

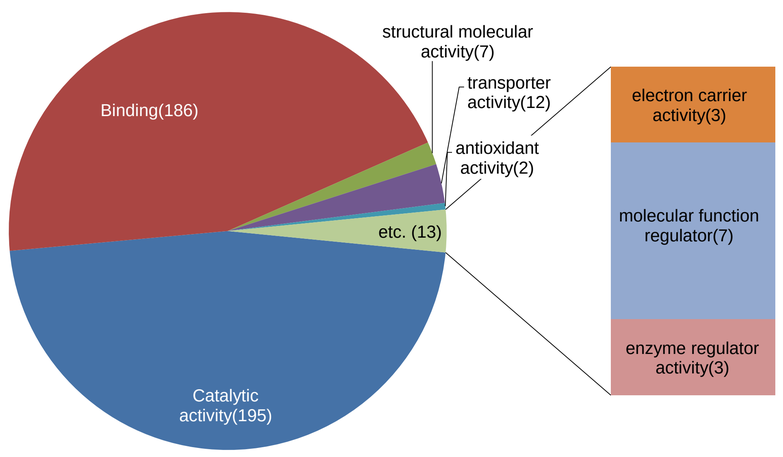


Fig 3. Functional annotations of the *N. fowleri* trophozoites and cysts based on Gene Ontology (GO) categories. The pie charts show the general categories of biological process (A), cellular component (B) and molecular function (C).

Table 4. The list of gene that showed upregulated proteins in *N. fowleri* cysts and trophozites.

Upregulated proteins in *N. fowelri* cyst	logFC
Uridine kinase	13.526
Calpain-5	8.641
Translin-associated factor X-interacting protein	7.394
Gag-Pol polyprotein	6.912
Profilin	6.813
Probable E3 ubiquitin-protein ligase	6.707
Serine/threonine-protein kinase	6.683
LisH domain-containing protein	5.032
Phospholipid-transporting ATPase	4.970
EF-hand domain-containing family member C2	4.579
Kinesin-like calmodulin-binding protein	4.429
Probable glycerol-3-phosphate dehydrogenase(mt)	4.327
Sphingosine-1-phosphate lyase	4.285
Nitrile-specifier protein	4.174
Upregulated proteins in *N. fowelri* trophozoites	logFC
Luminal-binding protein	14.309
Lysosomal Pro-X carboxypeptidase	7.017
Chaperone protein	4.349
12-oxophytodienoate reductase 1	4.344
Probable alpha-L-glutamate ligase	4.181
fatty acid desaturase	4.120
Cytoskeleton-associated protein	3.280
Tubulin alpha-6 chain	2.659
Actin	2.115
Microtubule-associated protein	1.996

CONCLUSIONS

- **This study is the first NGS-base method (RNA-sequencing) to set up transcriptomic database comparison between *N. fowleri* cysts and trophozoites.**
- **This analysis of difference expression genes between trophozoites and cysts could provide new insights into development, survival, environmental resistant factor, pathogenic related factor and protease of *N. fowelri*.**