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Differentially expressed genes of *Acanthamoeba castellanii* during encystation

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Abstract: To examine the expressed gene profile during encystation of *Acanthamoeba castellanii* Castellani, we used differentially expressed gene (DGE) screening by RT-PCR with 20 sets of random primers. From this analysis, we found that approximately 16 genes showed upregulation during encystation. We chose 6 genes, which had relatively higher expression levels, for further investigation. Based on homology search in database, DEG2 showed 55% of similarity with xylose isomerase, DEG9 showed 37% of similarity with Na P-type ATPase, and DEG14 showed 77% of similarity with subtilisin-like serine proteinase. DEG3 and DEG26 were identified as hypothetical proteins and DEG25 exhibited no significant similarity to any known protein. Encystation of *Acanthamoeba* has been suggested to be a process to resist adverse environmental or nutritional conditions. Further characterization studies of these genes may provide us with more information on the encystation mechanism of *Acanthamoeba*.

Key words: *Acanthamoeba*, differentially expressed gene, encystation

Acanthamoeba is an opportunistic pathogen responsible for several diseases in humans, such as granulomatous amebic encephalitis (GAE), amebic keratitis and dermatitis (Marciano-Cabral and Carbral, 2003). *Acanthamoeba* comprises 2 distinct stages, i.e., trophozoite and cyst. The trophozoite is the vegetative form, which conducts movement, phagocytosis, metabolism, and cell division (Baumann and Murphy, 1995; Wang and Ahearn, 1997). The cyst is formed from a trophozoite, when desiccation, starvation, or other adverse condition prevails, and is conversed to the trophozoite, when conditions are favorable (Weisman, 1976; Cordingley et al., 1996). Because trophozoites can transform to cysts under adverse conditions, such as host immune responses or chemical treatments in human infections (McClellan et al., 2002), it is difficult to treat the ameba infection with almost all kinds of antibiotics. If it is possible to understand the molecular mechanisms of encystation and to disrupt its process, it will be easier to treat the infection. It will also be applicable to drug development against other cyst forming pathogenic protozoa. To compare the gene expression between trophozoites and cysts, we conducted differentially expressed gene screening with *Acanthamoeba castellanii* Castellani (American Type Culture Collections; ATCC #30011, Beltsville, Maryland, USA).

Acanthamoeba was obtained from ATCC and was cultured axenically in PYG medium and induced encystation by the procedure of Bowers and Korn (1969). The total RNAs were purified from tropho-

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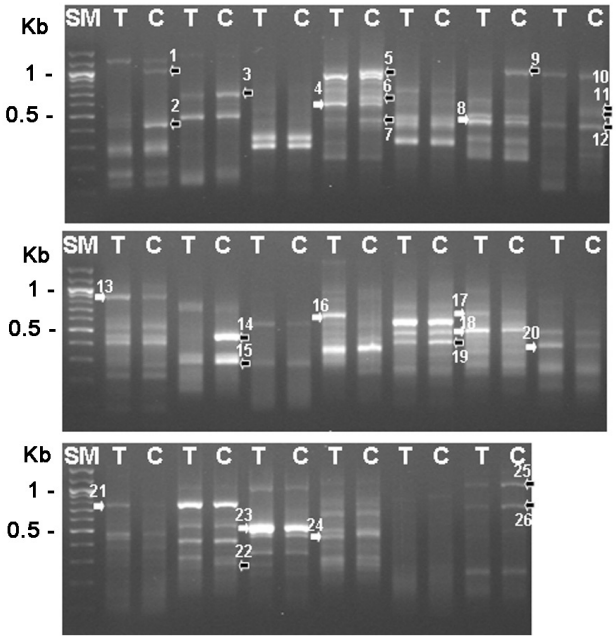

Fig. 1. Gel electrophoresis analysis with differentially expressed gene screening results (26 genes). SM; 100 bp ladder, T; trophozoite, C; 3-day cyst, black arrows; reduced genes during encystation, white arrows; increased genes during encystation.

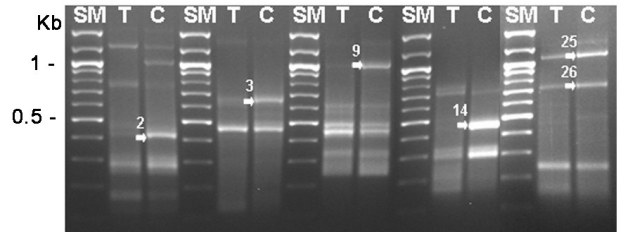

Fig. 2. Re-amplification of DEG2, 3, 9, 14, 25 and 26 between cysts and trophozoites. SM; 100 bp ladder, T; trophozoite, C; 3-day cyst, arrows; increased genes during encystation.

zoites and 3-day cysts, and their cDNAs were made, which was followed by the differentially expressed gene (DEG) screening with 20 sets of random primers (Seegen, Seoul, Korea). From this analysis between trophozoites and cysts, we found that approximately 16 genes were up-regulated and 10 genes were down-regulated during encystation (Fig. 1). Then, 6 genes (DEG2, 3, 9, 14, 25 and 26) showing significant up-regulation were confirmed the differential expression between cysts and trophozoites by DEG analysis again (Fig. 2). The expression pattern of these genes may suggest roles of the gene products in the encystation of *Acanthamoeba*. The genes were sequenced followed by submission for blast search (Table 1). Based on blast search results, the DEG2 protein showed 55% sequence similarity with xylose isomerase, DEG9 showed 37% similarity with Na P-type ATPase, DEG14 showed 77% similarity with subtilisin-like serine proteinase. DEG3 and DEG26 were identified as hypothetical proteins and DEG25 exhibited no significant similarity to any known protein at database.

Interestingly, DEG14 showed significant sequence similarity with subtilisin-like serine proteinase

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                *        20         *        40         *        60         *        80
DEG14 : MRAYYLVSACLLAVMLASASATLAPLHMAFDNQKVQNEYIVVMKPDMLHTQFNAYVGDIKAMFANQVSGDIINAMNVGNSFRALHVKT :  88
AhSub : MRAYF-VGACLLALLCASALASLAPLHRAIDAQRVENEYIVVMKPHVVHAEWTSYYSDIKLMFATQVQGDILQEWTIGNNFKAIHVKT :  87
        MRAY5 V ACLLA66 ASA A3LAPLH A D Q4V2NEYIVVMKP 66H 25  YV DIK MFA QV GDI6  W 6GN F4A6HVKT

         *       100         *       120         *       140         *       160         *
DEG14 : SPDDIHFFRTHPAVELIEENQVFHISSAYKTANCQRQDSAIWNLQRINTHPITGMDGDYSYNQEASDVDAYIIDTGILTTHQEFQ-GR : 175
AhSub : SEYAVNFFRTHPAVEFVEENQVVSISSAQ---NGLSQSGAIWNLQRINAQAISGMDGKYTYGTEAADVDAYIVDTGILTTHQEFDNGR : 172
        SP  6 FFRTHPAVE 6EENQV  ISSA    NC  Q  AIWNLQRIN   I3GMDG Y3Y  EA DVDAYI6DTGILTTHQEF  GR

         180         *       200         *       220         *       240         *       260
DEG14 : AIWGANFADAKDTDCNGHGTHVAGTVGGHTVGIARGVTLIAVKVLDCGGSGTTAGVISGIQWAGENARKRGRRSVANMSLGGGYSATL : 263
AhSub : ALWGANFADSQDTDCNGHGTHVAGTVGGKTVGIARGVTLYAVKVLGCDGSGTTNGVISGIQWVAENARKRGRKSVANMSLGGGYSSSL : 260
        A6WGANFAD  DTDCNGHGTHVAGTVGG TVGIARGVTL AVKVL C GSGTT GVISGIQW  ENARKRGR4SVANMSLGGGYS 3L

            *       280         *       300         *       320         *       340         *
DEG14 : NRAVASVVDAGVPFAVAAGNEDQNACNTSPASEAKAITVGATYFKGNYTQDTRAYFSNWGTCVDILAPGQSIKSAWIGSNSAYNTISG : 351
AhSub : NRAVASVVDAGVPFAVAAGNEDQNACNTSPASEAKAITVGATYYSGNFAQDIRASFSNWGTCVDILAPGQSIKSAWIGSNSAYNTISG : 348
        NRAVASVVDAGVPFAVAAGNEDQNACNTSPASEAKAITVGATY5 GN5 QD RA FSNWGTCVDILAPGQSIKSAWIGSNSAYNTISG

            360         *       380         *       400         *
DEG14 : TSMASPHVCGVAALVYGANPSYTAEQVKQQILQDTTAGVYELDCTKSGCSSTPNKMLYTARC : 413
AhSub : TSMASPHVCGVAALTFSANPSYSAADVKSKILADSTDNVIDIRCTRSGCSSTPNKLLYSARC : 410
        TSMASPHVCGVAAL 5 ANPSY3A  VK  IL D3T  V6 L CT4SGCSSTPNK6LY3ARC
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Fig. 3. Comparision of the deduced amino acid sequences of DEG14 with AhSub (subtilisin-like serine proteinase).

Table 1. Significant matches of DEGs with database sequences of other organisms

DEG No.	Length	Putative identification	Organism	Accession No.	Database	Score	*P*-value
DEG2	314	Xylose isomerase	*Reinekea* sp.	ZP 01113810.1	ref	150	$0.0 \times e^{-9}$
DEG3	596	Hypothetical protein	*Nitrosospira multiformis*	YP_412886.1	ref	157	$2.0 \times e^{-9}$
DEG9	701	Putative Na P-type ATPase	*Physcomitrella patens*	CAD91920.1	emb	283	$9.0 \times e^{-24}$
DEG14	383	Subtilisin-like serine proteinase	*Acanthamoeba healyi*	AF221523_1	gb	375	$7.0 \times e^{-35}$
DEG25	917	No significant similarity found					
DEG26	623	Hypothetical protein	*Rhodobacter sphaeroides*	YP_001167477.1	ref	100	$1.1 \times e^{-2}$

(AhSub), which was reported to be a secretory enzyme and a potential virulence factor (Hong et al., 2000). Until now, many proteinases of *Acanthamoeba*, such as serine, cysteine, and metallo-proteinases, have been suggested to be involved in the pathogenesis or phagocytosis (Mitro et al., 1994; Hong et al., 2000; Kong et al., 2000; Hong et al., 2002; Kim et al., 2006; Serrano-Luna et al., 2006). We identified full length ORF of DEG14 of *A. castellanii* by RACE-PCR. Based on the sequence analysis, DEG14 belonged to the serine protease family subtilisin group (data not shown). The deduced amino acid sequence of DEG14 showed 76% similarity with that of AhSub (Fig. 3). Here, we identified 6 genes induced by encystations, while its expression in the trophozoite is very low or absent. Further studies on these genes may provide more understanding on the encystation mechanisms of *Acanthamoeba*.

REFERENCES

Baumann O, Murphy DB (1995) Microtubule-associated movement of mitochondria and small particles in *Acanthamoeba castellanii*. *Cell Motil Cytoskel* **32:** 305-317.

Bowers B, Korn ED (1969) The fine structure of *Acanthamoeba castellanii* (Neff strain). II. Encystment. *J Cell Biol* **41:** 786-805.

Cordingley JS, Wills RA, Villemez CL (1996) Osmolarity is an independent trigger of *Acanthamoeba castellanii* differentiation. *J Cell Biochem* **61:** 167-171.

Hong YC, Hwang MY, Yun HC, Yu HS, Kong HH, Yong TS, Chung DI (2002) Isolation and characterization of a cDNA encoding a mammalian cathepsin L-like cysteine proteinase from *Acanthamoeba healyi*. *Korean J Parasitol* **40:** 17-24.

Hong YC, Kong HH, Ock MS, Kim IS, Chung DI (2000) Isolation and characterization of a cDNA encoding a subtilisin-like serine proteinase (ahSUB) from *Acanthamoeba healyi*. *Mol Biochem Parasitol* **111:** 441-446.

Kim WT, Kong HH, Ha YR, Hong YC, Jeong HJ, Yu HS, Chung DI (2006) Comparison of specific activity and cytopathic effects of purified 33 kDa serine proteinase from *Acanthamoeba* strains with different degree of virulence. K*orean J Parasitol* **44:** 321-330.

Kong HH, Kim TH, Chung DI (2000) Purification and characterization of a secretory serine proteinase of *Acanthamoeba healyi* isolated from GAE. *J Parasitol* **86:** 12-17.

Marciano-Cabral F, Cabral G (2003) *Acanthamoeba* spp. as agents of disease in humans. *Clin Microbiol Rev* **16:** 273-307.

McClellan K, Howard K, Mayhew E, Niederkorn J, Alizadeh H (2002) Adaptive immune responses to *Acanthamoeba* cysts. *Exp Eye Res* **75:** 285-293.

Mitro K, Bhagavathiammai A, Zhou OM, Bobbett G, McKerrow JH, Chokshi R, Chokshi B, James ER (1994) Partial characterization of the proteolytic secretions of *Acanthamoeba polyphaga*. *Exp Parasitol* **78:** 377-385.

Serrano-Luna Jde J, Cervantes-Sandoval I, Calderon J, Navarro-Garcia F, Tsutsumi V, Shibayama M (2006) Protease activities of *Acanthamoeba polyphaga* and *Acanthamoeba castellanii*. *Can J Microbiol* **52:** 16-23.

Wang X, Ahearn DG (1997) Effect of bacteria on survival and growth of *Acanthamoeba castellanii*. *Curr Microbiol* 34: 212-215.

Weisman RA (1976) Differentiation in *Acanthamoeba castellanii*. *Annu Rev Microbiol* **30:** 189-219.