

## 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 converted to the trophozoite, when conditions are favorable (Weisman,

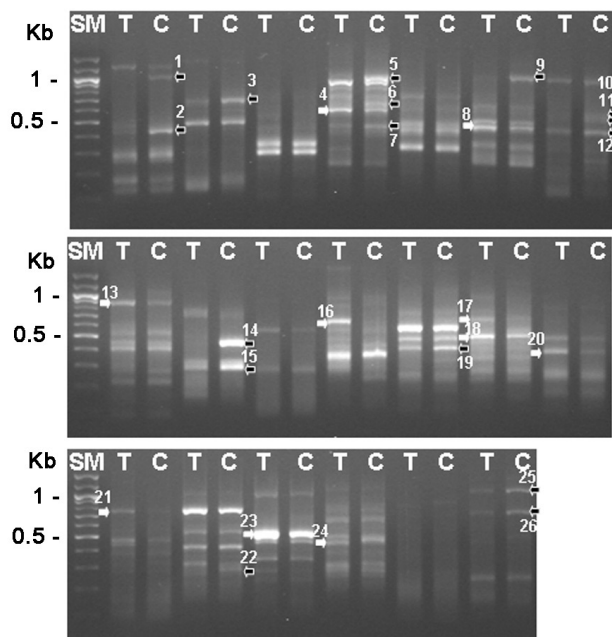
1976; Cordingley et al., 1996). Because trophozoites can transform to cysts under adverse conditions, such as host immune responses or chemical treatments in human infections (McClellan et al., 2002), it is difficult to treat the ameba infection with almost all kinds of antibiotics. If it is possible 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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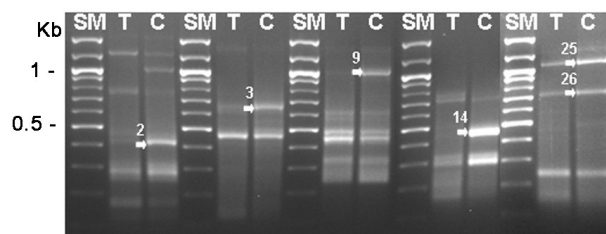
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**Fig. 1.** Gel electrophoresis analysis with differentially expressed gene screening results (26 genes). SM; 100 bp ladder, T; trophozoite, C; 3-day cyst, black arrows; reduced genes during encystation, white arrows; increased genes during encystation.

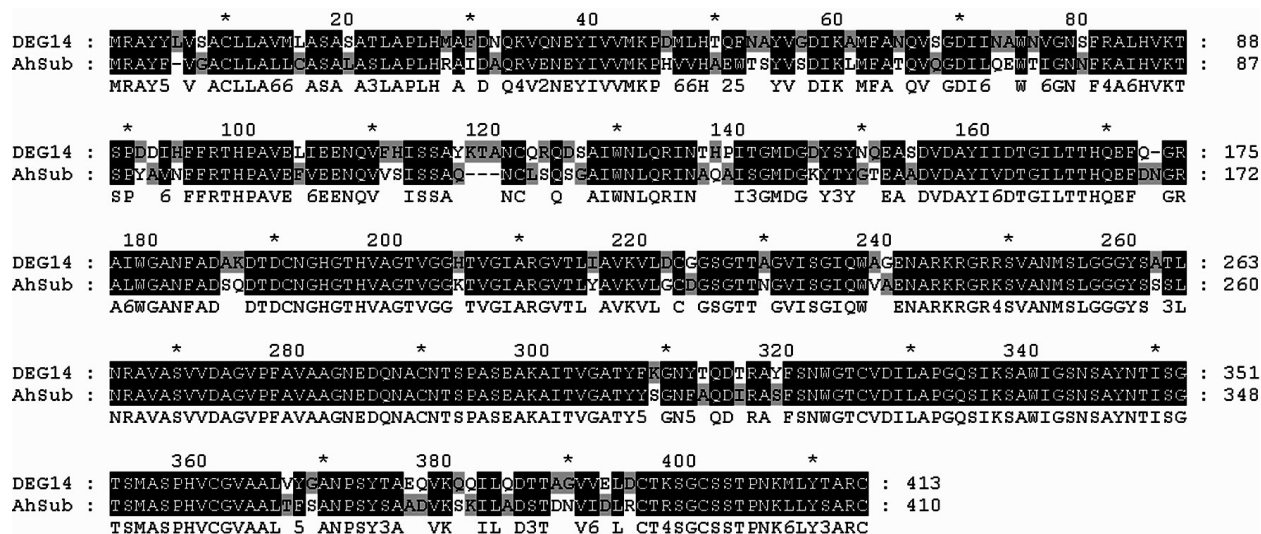
zoites and 3-day cysts, and their cDNAs were made, which was followed by the differentially expressed gene (DEG) screening with 20 sets of random primers (Seegen, Seoul, Korea). From this analysis between trophozoites and cysts, we found that approximately 16 genes were up-regulated and 10 genes were down-



**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.

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



**Fig. 3.** Comparison 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	<i>Reinekea</i> sp.	ZP_01113810.1	ref	150	$0.0 \times e^{-9}$
DEG3	596	Hypothetical protein	<i>Nitrosospira multififormis</i>	YP_412886.1	ref	157	$2.0 \times e^{-9}$
DEG9	701	Putative Na P-type ATPase	<i>Physcomitrella patens</i>	CAD91920.1	emb	283	$9.0 \times e^{-24}$
DEG14	383	Subtilisin-like serine proteinase	<i>Acanthamoeba healyi</i>	AF221523_1	gb	375	$7.0 \times e^{-35}$
DEG25	917	No significant similarity found					
DEG26	623	Hypothetical protein	<i>Rhodobacter sphaeroides</i>	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*.

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