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Expressed sequence tags (ESTs) analysis of *Acanthamoeba healyi*

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Abstract: Randomly selected 435 clones from *Acanthamoeba healyi* cDNA library were sequenced and a total of 387 expressed sequence tags (ESTs) had been generated. Based on the results of BLAST search, 130 clones (34.4%) were identified as the genes enconding surface proteins, enzymes for DNA, energy production or other metabolism, kinases and phosphatases, protease, proteins for signal transduction, structural and cytoskeletal proteins, cell cycle related proteins, transcription factors, transcription and translational machineries, and transporter proteins. Most of the genes (88.5%) are newly identified in the genus *Acanthamoeba*. Although 15 clones matched the genes of *Acanthamoeba* located in the public databases, twelve clones were actin gene which was the most frequently expressed gene in this study. These ESTs of *Acanthamoeba* would give valuable information to study the organism as a model system for biological investigations such as cytoskeleton or cell movement, signal transduction, transcriptional and translational regulations. These results would also provide clues to elucidate factors for pathogenesis in human granulomatous amoebic encephalitis or keratitis by *Acanthamoeba*.
Key words: *Acanthamoeba healyi*, expressed sequence tags, novel genes

INTRODUCTION

The genus, *Acanthamoeba*, a human pathogen causing granulomatous amoebic encephalitis (GAE) and amebic keratitis (Sisson et al., 1994), has been known to be a vector for pathogenic microbes such as *Mycobacterium* spp., *Listeria* spp., and *Legionella* spp. (Jadin, 1973; Ly & Muller, 1990; Field, 1991). In addition to these medical importances, it is also well known that *Acanthamoeba* is a good model system to study eukaryotic cell biology due to its relatively large size, rapid growth in culture, active motility, and well developed cytoskeleton (Byers et al., 1990).

Although the ploidy and the total DNA content of the genus *Acanthamoeba* are unclear at the present, Byers et al. (1990) speculated that the genome size of the amoeba would be ~1×10^8 bp of which the size could express more than 5,000 transcripts; however, only a few genes and proteins have been reported. Most of the genes identified are 18s rDNA sequences for the taxonomic purpose (Gast et al., 1996; Stothard et al., 1998), actin, myosins and actin binding proteins to study cytoskeleton (Nellen & Gallwitz, 1982; Cooper

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et al., 1986; Jung et al., 1989; Pollard & Rimm, 1991; Kelleher et al., 1995; Lee et al., 1999), and mitochondrial genome (Burger et al., 1995). Therefore most genes of *Acanthamoeba* have yet to be uncovered.

Characterization of the transcribed genes in a certain organism by expressed sequence tag (EST) analysis, single pass sequencing of randomly selected cDNA clones has been applied to many organisms including parasitic protozoa such as *Plasmodium falciparum* (Chakrabarti et al, 1994), *Trypanosoma brucei rhodesiense* (El-Sayed et al., 1995), *Toxoplasma gondii* (Wan et al., 1996; Manger et al., 1998), and *Entamoeba histolytica* (Azam et al., 1996; Tanaka et al., 1997). In addition to the candidate genes for pathogenic factors, many novel genes for stage specific, cell cycle regulatory, or related to cell signaling were identified in these protozoa by EST analysis.

ESTs analysis of *Acanthamoeba* could characterize the expression pattern of the genes, providing invaluable information to understand the genetics and the identification of many novel genes in this genus.

In the present study, we report the results of ESTs analysis of *A. healyi* OC-3A strain isolated from the brain of a GAE patient.

MATERIALS AND METHODS

Amoeba culture

An isolate of *Acanthamoeba* from the brain of a GAE patient, *A. healyi* OC-3A, was obtained from ATCC and cultured in Proteose peptone-Yeast extract-Glucose medium at 25°C.

RNA preparation

Trophozoites of *A. healyi*, washed with phosphate- buffered saline (PBS), were homogenized with β-mercaptoethanol and RNA denaturation solution (Stratagene, San Diego, CA, USA). One milliliter of 3 M sodium acetate (pH 4.0) was added to the solution and mixed by inversion. Water saturated phenol was added and mixed well with the solution. The mixture was shaken vigorously after adding chloroform/isoamyl alcohol (24:1), and then was incubated on ice for 15 min. The supernatant of the mixture after centrifugation was moved to a new tube and incubated with 1 volume of isopropanol at –20°C for 1 hr for precipitation of the RNA. The RNA pellet was dissolved with the RNA denaturation solution and reprecipitated with equal volume of isopropanol. DEPC treated Q-water was added to the ethanol washed and vacuum dried RNA pellet. mRNA was purified from total RNA sample using Poly (A) Quick mRNA isolation kit (Stratagene, San Diego, CA, USA).

Construction of cDNA library

A unidirectional oligo (dT)-primed EcoRI/Xho I cDNA library was constructed in UNI-ZAP™ (Stratagene, San Diego, CA, USA). Briefly, first strands, synthesized by reverse transcription of mRNA of *A. healyi*, were used to make second strands by the action of RNase H and DNA polymerase I. cDNA with blunted termini were ligated with *Eco* RI adapters and digested with Xho I. After size fractionation, cDNA over the size 400 bp were packaged into UNI-ZAPTM XR vector arms by ligation. Packaged cDNAs were incubated with the host cell of XL1-blue MRF strain on agarose LB medium. More than 5 millions clones of this library were amplified and the aliquots of the library were stored at 4°C until use.

Sequencing of randomly selected cDNA clones

cDNA library mixture were incubated with XL1-Blue MRF cell to allow in vivo excision using ExAssist helper phage, transfected into *Escherichia coli* SOLR strain, and plated on LB-ampicillin agar plates. Each randomly selected colony from the plates was inoculated into LB-ampicillin broth and incubated at 37°C overnight. Plasmid with the cDNA insert was extracted with plasmid DNA purification system (Wizard® Plus Minipreps, Promega, USA). The size of cDNA inserts obtained by digestion of the plasmid DNA with EcoR I and Xho I were estimated by electrophoresis on agarose gel with Hind III digested λ phage DNA, a DNA size standard. Alkaline denaturation of the plasmid DNA and the dideoxy chain termination method using DNA sequencing kit (T7 Sequenase version 2.0, Amersham, USA) and ^{35}S dATP were applied to elucidate the sequence data of the randomly

selected cDNA clones. After electrophoresis of the reaction samples for sequencing, the vacuum dried acrylamide gel was exposed on X-ray film. Sequence data were edited to remove vector and ambiguous sequences, and less than 100 bases were also rejected.

Basic Local Alignment Search Tool (BLAST) search

The sequence data of cDNA clones by random partial sequencing were subjected to examine similarities in the nucleic acid and protein databases using the BLAST on the National Center for Biotechnology Information (NCBI). The cDNA sequences were compared against nucleotide data by the program BLASTN, and the conceptual translation products of query sequences against translated nucleic acid and protein databases by BLASTX. Matches were considered to be significant only when the probability (P) was less than 0.0001 and scores were >160 for BLASTN and >80 for BLASTX.

RESULTS

Among 435 cDNA clones sequenced, the sequence data of 378 clones were submitted for blast search (Table 1). It was 130 clones (34.4%) identified by high homology with the DNA sequence of *Acanthamoeba* or other organisms in the public data base. Although 15 clones were matched with *Acanthamoeba* genes already studied, they were just 3 kinds of genes and twelve of them were the actin gene, the most commonly found gene in this study. The other 115 clones (88.5%, 94 different genes) are reported in the present paper for the first time in this genus.

Table 1. *Acanthamoeba healyi* cDNA library sequencing

EST category	No. of clones
Total clones sequenced	435
ESTs submitted for BLAST search	378
ESTs identified by homology	130
Unique ESTs identified	94
ESTs with homology to *Acanthamoeba* genes	15
Redundant ESTs	20
Common ESTs	
actin	12
60S acidic ribosomal protein PO	4
elongation factor 1 alpha	3
major vault protein	3

Based on the results of BLAST search, ESTs with predicted or known functions were classified into putative cellular roles (Table 2). They were 4 clones for surface protein, 2 clones for DNA metabolism, 12 clones for energy metabolism, 10 clones for kinase and phosphatase, 17 clones for other metabolism, 1 clone for protease, 10 clones for signal transduction, 18 clones for structure and cytoskeleton, 3 clones for cell cycle related proteins, 5 clones for transcription factor, 32 clones for transcription and translational machinery, 4 clones for transporters, and 12 clones for not classified.

DISCUSSION

It is the first time to analyze ESTs of *Acanthamoeba* known as an human pathogen and a good model system for biological studies. The most frequently presented gene was actin gene which appeared 12 times. This was as expected because *Acanthamoeba* has well developed cytoskeleton and move very actively (Pollard, 1982). The biggest class among identified clones was genes for protein synthesis with the number of 32 from 130 clones. Similar results were reported in the EST analysis of *Entamoeba histolytica*, *Typanosoma cruzi*, and *E. dispar* (Tanaka et al., 1997; Verdun et al., 1998; Sharma et al., 1999).

Among four clones of genes for surface protein, two were identified as non-integrin type laminin binding protein. Adhesion would be the very first and important step to infect host by tissue invading parasites. Laminin binding proteins has been reported from several parasitic organisms including *Trichomonas vaginalis* (Silva Filho et al., 1988), *Trypanosoma brucei rhodesiense* (gene bank, W99296), *Leishmania donovani* (Ghosh et al., 1996) and *Echinococcus granulosus* (Zhang et al., 1997). The ability to recognize extracellular matrix proteins such as laminin or fibronectin

Table 2. Significant matches of *Acanthamoeba healyi* ESTs with database sequences from other organisms

Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
Surface protein							
Ah161	208	coatomer delta subunit	*Oryza sativa*	sp[a)]	P49661	182	3.80E-17
Ah217	275	laminin receptor like protein (P40)	*Daucus carota*	dbj[b)]	AB012702	214	3.10E-21
Ah477	256	laminin receptor like protein (P40)	*D. carota*	dbj	AB012702	202	1.20E-19
Ah489	202	70 kD peroxisomal membrane protein	*Mus musculus*	sp	P55096	145	4.30E-12
DNA metabolism							
Ah468	206	methyl transferase	*Saccharomyces cerevisiae*	sp	P25087	226	2.60E-23
Ah534	308	double-strand break repair protein	*Caenorhabditis elegans*	gb[c)]	U40029	236	3.50E-24
Energy metabolism							
Ah015	153	acyl-CoA dehydrogenase	*C. elegans*	emb[d)]	AL032621	102	1.40E-06
Ah029	227	ATP synthase gamma chain	*Arabidopsis thaliana*	sp	Q01908	89	0.00034
Ah035	180	3-hydroxyisobutyrate dehydrogenase	Rabbit	sp	P32185	107	5.50E-07
Ah089	262	adenylosuccinate synthetase precursor	*A. thaliana*	sp	Q96529	217	1.00E-21
Ah110	261	NADP-isocitrate dehydrogenase	*Glycine max*	gb	AF095445	286	3.20E-31
Ah335	256	NADP-isocitrate dehydrogenase	Pig	gb	M86719	523	1.80E-33
Ah452	193	6-phosphogluconate dehydrogenase	*Cunninghamella elegans*	emb	Y17297	140	1.90E-11
Ah475	181	fatty acid synthase, subunit alpha	*Candida albicans*	sp	P43098	206	1.20E-20
Ah532	364	NADH-ubiquinone oxidoreductase	*Homo sapiens*	sp	Q16795	277	1.00E-29
Ah542	281	enolase	*Spongilla* sp.	gb	U85829	198	5.00E-19
Ah577	254	fatty acid synthase, subunit alpha	*C. albicans*	sp	P43098	135	5.40E-23
Ah595	137	transketolase	*Spinacia oleracea*	gb	L76554	127	5.50E-10
Immunology							
Ah342	228	immunoglobulin heavy chain V-D-J region	*Oryctolagus cuniculus*	gb	AF058603	77	0.015
Ah464	244	Leukotriene A-4 hydrolase	*Cavia porcellus*	sp	P19602	110	4.90E-07
Kinase and phosphatase							
Ah042	225	cytohesin 2	*H. sapiens*	emb	Z94160	100	1.00E-05
Ah066	271	mevalonate kinase	Rat	sp	P17256	100	2.10E-06
Ah119	243	c2 domain	*H. sapiens*	gb	AC005278	177	2.90E-16
Ah172	269	3-phosphoglycerate kinase	*Thermotoga maritime*	emb	X75437	254	8.90E-27
Ah177	160	myosin heavy chain kinase B	*Dictyostelium discoideum*	sp	P90648	91	1.30E-07
Ah233	232	nucleoside diphosphate kinase 3	*A. thaliana*	gb	AF044265	227	9.30E-23
Ah401	119	S-phase kinase associated protein like	*D. discoideum*	gb	U73686	90	9.50E-05
Ah436	207	Skb1Hs	*H. sapiens*	gb	AF015913	167	4.40E-15
Ah463	223	phosphatases pleiotropic regulator	*A. thaliana*	sp	Q39190	280	1.40E-30

Table 2. Continued

Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
Ah482	242	phosphoenolpyruvate carboxykinase	*Chlorobium limicola*	sp	Q08262	116	7.30E-08
Ah584	272	protein kinase	*Schizosaccharomyces pombe*	emb	AL022245	80	8.90E-03
Other metabolism							
Ah030	208	ADP-ribosylglycohydrolase	*Methanococcus jannaschii*	pir[e)]	C64448	83	0.0017
Ah039	237	phosphomannomutase	*C. elegans*	emb	AL021481	84	2.00E-03
Ah043	190	ubiquitin	*Acanthamoeba castellnii*	pir	S45304	252	6.30E-27
Ah090	217	thioredoxin peroxidase	*Trypanosoma brucei rhodesiense*	sp	Q26695	213	2.30E-21
Ah111	293	protein disulfide isomerase	*D. discoideum*	gb	AF019112	245	1.80E-25
Ah162	214	dihydrolipoamide dehydrogenase	garden pea	pir	A42494	99	1.10E-05
Ah171	268	thioredoxin peroxidase	Pig	sp	P52552	117	4.00E-09
Ah173	224	ubiquitin fusion degradation protein 1	*M. musculus*	sp	P70362	112	5.70E-09
Ah174	185	protein disulfide isomerase	*Drosophila melanogaster*	sp	P54399	168	3.30E-15
Ah179	275	uricase	*Sus scrofa*	sp	P16164	61	1.20E-06
Ah346	236	proteosome subunit DD5	*D. discoideum*	sp	P34120	555	3.50E-36
Ah381	260	20S proteosome subunit PAC1	*A. thaliana*	gb	AF043521	335	5.50E-38
Ah421	151	glutamate decarboxylase	*D. melanogaster*	sp	P20228	117	1.20E-08
Ah458	150	ubiquitin	*A. castellnii*	sp	P49634	148	6.60E-13
Ah461	229	proteosome subunit DD5	*D. discoideum*	sp	P34120	310	1.10E-34
Ah581	228	ELAV protein (RNA metabolism)	*D. melanogaster*	sp	P16914	102	5.50E-06
Ah582	190	thiazole mono	*Escherichia coli*	sp	Q46948	97	2.30E-07
Protease							
Ah068	195	aminopeptidase-like protein	*A. thaliana*	emb	Z99708	104	1.80E-06
Signal transduction							
Ah040	260	GTP binding protein (rab2)	*Chlamydomonas reinhardtii*	sp	Q39570	187	1.40E-17
Ah155	250	ras related protein RAB4	*D. discoideum*	sp	P36410	386	4.60E-45
Ah184	284	visinin like protein 3 (VILIP-3)	*H. sapiens*	sp	P37235	67	2.00E-05
Ah348	182	Guanine nucleotide binding protein	*Hydra vulgaris*	sp	Q25189	143	5.90E-12
Ah487	185	14-3-3 protein	*D. melanogaster*	sp	P92177	198	8.30E-19
Ah501	170	14-3-3 protein	*D. melanogaster*	sp	P92177	183	1.20E-16
Ah503	283	rac GTP binding protein Arac10	*A. thaliana*	gb	AF079485	275	1.20E-29
Ah578	246	small GTP binding protein Rab-7	*H. sapiens*	gb	U44104	188	8.90E-18
Ah603	307	GTP binding nuclear protein SPI1	*S. pombe*	sp	P28748	386	7.00E-45
Ah622	251	G protein beta subuit like	*M. musculus*	pir	I49700	288	3.80E-31
Ah627	255	G protein beta subuit like	*M. musculus*	pir	I49700	299	5.40E-33

Table 2. Continued

Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
Structural and cytoskeletal							
Ah016	225	F-actin capping protein bets subunit	*D. discoideum*	sp	P13021	220	2.80E-22
Ah031	195	actin	*A. castellanii*	sp	P02578	280	9.90E-31
Ah072	245	actin-bundling protein	*Physarum polycephalum*	pir	S32566	93	2.50E-12
Ah145	217	actin	*A. castellanii*	sp	P02578	337	1.80E-38
Ah215	286	actin	*A. castellanii*	sp	P02578	425	2.60E-50
Ah219	173	actin-related protein 2 (Arp2)	*A. castellanii*	sp	P53487	197	1.10E-19
Ah220	192	gelation factor	*D. discoideum*	sp	P13466	162	3.30E-14
Ah221	268	actin	*A. castellanii*	sp	P02578	384	1.40E-44
Ah231	271	tubulin alpha 1	*O. sativa*	sp	P28752	297	1.00E-32
Ah341	275	actin	*A. castellanii*	sp	P02578	296	4.50E-38
Ah344	197	actin	*A. castellanii*	sp	P02578	227	2.90E-30
Ah354	159	actin	*A. castellanii*	sp	P02578	233	1.20E-24
Ah361	287	actin	*A. castellanii*	sp	P02578	461	2.80E-55
Ah388	270	actin	*A. castellanii*	sp	P02578	469	2.00E-56
Ah402	167	actin	*A. castellanii*	sp	P02578	277	1.00E-30
Ah499	295	actin	*A. castellanii*	sp	P02578	498	2.30E-60
Ah599	164	actin	*A. castellanii*	sp	P02578	260	2.30E-28
Ah614	211	coronin (actin binding protein)	*D. discoideum*	sp	P27133	164	1.20E-14
Cell cycle related							
Ah114	193	prohibitin	*Toxocara canis*	gb	U97204	100	5.90E-06
Ah151	234	diaphanous 1	*H. sapiens*	gb	AF051782	131	6.00E-10
Transcription factors							
Ah147	263	myocyte-enhancer-binding factor 2	*D. melanogaster*	emb	X83527	114	4.40E-13
Ah163	230	myocyte-enhancer-binding factor 2	*Cyprinus carpio*	dbj	AB012884	85	1.10E-10
Ah235	271	SNF4-like protein (Pv42p)	*Phaseolus vulgaris*	gb	U40713	90	3.60E-04
Ah356	150	dribble protein	*D. melanogaster*	emb	Z96931	151	2.50E-13
Ah396	205	dribble protein	*D. melanogaster*	emb	Z96931	280	1.10E-30
Transcription and translational machinery							
Ah006	289	ribosomal protein L38	rat	emb	X57007	215	8.90E-08
Ah082	249	elongation factor 2	*C. elegans*	sp	P29691	106	1.80E-14
Ah085	266	elongation factor 1 gamma	*C. elegans*	sp	P54412	108	4.00E-07
Ah116	228	ribosomal protein L7	*H. sapiens*	pir	S30212	114	2.60E-07
Ah146	237	40S ribosomal protein S16	*Fritillaria agrestis*	sp	O22647	215	1.50E-21

Table 2. Continued

Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
Ah148	258	RNAse L inhibitor	*M. musculus*	gb	U90446	181	8.90E-17
Ah156	264	elongation factor 1-alpha	*Blastocystis hominis*	sp	P54959	335	5.50E-38
Ah181	288	ATP-dependent RNA helicase	*H. sapiens*	emb	Y14768	198	1.10E-20
Ah187	270	elongation factor 1B gamma	*O. sativa*	dbj	D89802	151	1.40E-12
Ah193	247	60S acidic ribosomal protein PO	*Bos taurus*	sp	Q95140	185	2.30E-17
Ah208	275	RNA binding protein (multiple splicing)	*H. sapiens*	dbj	D84107	114	1.90E-07
Ah232	167	40S ribosomal protein S14	*Maize*	sp	P19951	149	4.70E-13
Ah339	255	elongation factor 2	*Trypanosoma cruzi*	dbj	D50806	161	5.20E-14
Ah345	257	60S acidic ribosomal protein PO	*B. taurus*	sp	Q95140	185	2.60E-17
Ah347	209	elongation factor 1-alpha	*Bombyx mori*	sp	P29520	241	2.90E-25
Ah365	236	ribosomal protein S16	Mouse	sp	P14131	160	5.90E-14
Ah390	287	elongation factor 1 alpha	*B. mori*	sp	P29520	415	6.20E-49
Ah399	219	elongation factor 1 beta	*D. melanogaster*	emb	AL031863	86	7.80E-04
Ah420	134	ribosomal protein L41	*K. fragilis*	gb	M62394	154	7.90E-07
Ah448	200	tRNA splicing protein SPL1	*C. maltosa*	sp	P87187	146	3.10E-12
Ah454	222	60S ribosomal protein L14EB	*S. cerevisiae*	sp	P38754	136	3.30E-11
Ah460	160	60S acidic ribosomal protein PO	*Plasmodium falciparum*	sp	Q94660	91	4.70E-05
Ah493	301	40S ribosomal protein RS16	*S. cerevisiae*	sp	P40213	294	3.30E-32
Ah500	218	40S ribosomal protein S3A	*H. sapiens*	sp	P48154	135	1.40E-10
Ah511	204	40S ribosomal protein S7	*Avicennia marina*	gb	AF098519	149	1.20E-12
Ah514	207	60S acidic ribosomal protein PO	*P. falciparum*	sp	Q94660	145	4.80E-12
Ah575	219	elongation factor 1 beta	*D. melanogaster*	emb	AL031863	86	7.90E-04
Ah579	134	phenylalanyl-tRNA synthetase	*C. albicans*	emb	AL033503	120	4.90E-09
Ah615	211	60S ribosomal protein L14 (CAG-ISL 7)	*H. sapiens*	sp	P50914	163	1.60E-14
Ah616	240	ribosomal protein L32	*Kluveromyces lactis*	gb	L05772	174	1.80E-04
Ah621	255	splicing factor	*H. sapiens*	sp	Q08170	95	6.50E-05
Ah623	223	elongation factor 1 alpha	*B. mori*	sp	P29520	315	2.10E-35
Transporters							
Ah037	171	mitochondrial carrier protein DIF-1	*C. elegans*	pir	S55056	119	8.50E-10
Ah074	177	protein transport protein SEC13	*H. sapiens*	sp	P55735	87	4.10E-05
Ah528	241	adenine nucleotide translocator	*Lupinus albus*	emb	AJ003197	266	1.40E-28
Ah572	181	protein transport protein SEC23	*M. musculus*	pir	I60247	141	1.10E-11
Not classified							
Ah008	215	hypothetical 69.8 kD protein	*S. cerevisiae*	sp	Q06053	142	1.40E-11
Ah013	311	yeast UTR3 protein	*S. cerevisiae*	emb	Z72510	119	4.90E-08

Table 2. Continued

Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
Ah087	222	hypothetical 36.8 kD protein	*S. pombe*	sp	Q10169	93	8.70E-05
Ah335	214	Major vault protein alpha	*D. discoideum*	sp	P34118	198	3.30E-25
Ah384	196	hypothetical protein YER007c-a	*S. cerevisiae*	pir	S53543	99	2.50E-09
Ah446	169	hypothetical protein YER007c-a	*S. cerevisiae*	pir	S53543	101	3.40E-06
Ah457	240	SGT protein	*Rattus norvegicus*	emb	AJ222724	90	3.10E-05
Ah478	185	Nbr1	*M. musculus*	gb	U73039	102	2.90E-06
Ah486	291	Down syndrome critical region protein A	*H. sapiens*	sp	O14972	215	2.40E-21
Ah492	248	EST yk400g3.3	*C. elegans*	emb	Z81592	86	1.10E-03
Ah543	218	Major vault protein alpha	*D. discoideum*	sp	P34118	165	1.00E-14
Ah574	175	Major vault protein beta	*D. discoideum*	sp	P54659	137	2.10E-11
rRNA							
Ah197	256	large subunit ribosomal RNA	*Plumbago auriculata*	gb	AF036492	746	5.60E-71

Database abbreviation: [a)]sp, swissprot; [b)]dbj, Data Base of Japan; [c)]gb, GenBank; [d)]emb, EMBL; [e)]pir, Protein Informatio Resource.

has been known to correlate with invasiveness (Silva Filho et al., 1988; Ghosh et al., 1999). Further characterization of laminin binding protein in *A. healyi* would help to discover the mechanisms of the invasion by the amoeba.

A lot of genes for proteins involved in various metabolism were found in *Acanthamoeba* for the first time in this study except for the ubiquitin (Ub) gene (Hu & Henney, 1997). Six clones were identified to be associated with Ub-proteasome protein destruction system. Ub-proteasome pathway of intracellular proteolysis has been shown to be involved in various biologically important processes, such as the cell cycle, cellular metabolism, apoptosis, signal transduction, immune response, and protein quality control (Hilt and Wolf, 1996; Ciechanover, 1998; Tanaka, 1998).

Little information has been reported for signal transduction in genus *Acanthamoeba*. In this study, many kinds of cell signaling molecules, including Rabs, 14-3-3 protein and rac, were identified. Rabs regulate the flux through individual steps of the intracellular membrane trafficking pathway. The small GTPase Rab2 is a resident of pre-Golgi intermediates and required for protein transport from the endoplasmic reticulum to the Golgi complex (Tisdale et al., 1992). The GTP binding motif, GDTGVGKS, was conserved in the sequence of the clone Ahc040 identified as Rab2. A Rab protein isolated by EST analysis had been characterized in *Trypanosoma brucei* by Field et al. (1999).

In addition, gene for prohibitin known to negatively regulate cell proliferation in mammals was identified in *Acanthamoeba*. Further studies on this gene would give information in the regulation of *Acanthamoeba* proliferation and development. Three clones were identified as gene for vault protein showing high homology with that of *Dictyostelium discoideum*. Although vault proteins are found in nearly all eukaryotic cells, the function of the protein has yet to be elucidated.

Lots of the genetic information of *Acanthamoeba* obtained in this study would be very helpful to figure out pathogenetic mechanisms of GAE or keratitis by *Acanthamoeba* and to develop therapeutic reagents specific to the amoeba.

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