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Research Article

Insights from the cDNA and EST analysis of *Antrodia cinnamomea*

Jing Li[1], Yanling Liu[1], Richard Yankey[1, 2], Dongmei Lin[1] & Zhanxi Lin[1*]

[1]National Engineering Research Center of JUNCAO Technology, Fujian Agriculture and orestry University, Fujian, Fuzhou 350002, PR China; [2]Life Science College, Fujian Agriculture and Forestry University, Fujian, Fuzhou 350002, PR China; Zhanxi Lin – Email ID: lzxjuncao@163.com; *Corresponding author

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Abstract:
It is of interest to document the insights gleaned from the cDNA and EST analysis of *Antrodia cinnamomea* (a fungal species). Hence a library of sequences was constructed and analysed using standard procedures to gain new insights. Therefore, 65 ESTs, with size ranging from 300-2000 bp, were constructed. This included 46 ESTs with definite annotation, 18 ESTs were hypothetical and 1 new protein derived from BLAST analysis. We assigned 227 Gene Ontology terms linked to cell composition, transport, catalytic activity, and regulation functions in these sequences. Moreover, 56 matching genes were found in 8 Kyoto Encyclopedia of Genes and Genomes pathways. Data also showed 271 SSRs from *Antrodia cinnamomea* ESTs with an occurrence frequency of 96.82%. The STRING data analysis showed 29 genes encoded enzymes highly involved in protein-to-protein interactions linked to expression of regulation function. Thus, we documented some insights from the cDNA and EST analysis of *Antrodia cinnamomea* for further data mining.

Keywords: *Antrodia cinnamomea*; genome wide analysis; cDNA; EST; GO functional annotation;

Background:
Antrodia cinnamomea is a rare precious medical fungus found mainly in Taiwan China, which is well known for its immunomodulatory, anti-inflammation and anti-cancer pharmacological attributes **[1]**. Notably, triterpenoids and sterols are the most classical components and are biosynthesized via the meavalonic acid (MVA) pathway **[2]**. However, the relative genes and protein involved in the metabolite biosynthesis pathway of A. cinnamomea have rarely been studied. Less molecular information and EST sequences of A. cinnamomea were retrieved in the NCBI database. Complementary DNA (cDNA) library construction application for developing expressed sequence tags (ESTs) and transcriptome, was first reported in 1994 **[3]**. Gateway technology is a direct approach to speed-up functional open reading frame analysis **[4]**, and the cDNA fragment is directly recombined into the destination vector, which is highly efficient, simplified cloning, chimeric clones reduced, less size bias and easily clone to a large extent, accurately reflecting the expression level of the original abundance of mRNA in the library. Simple sequence repeats (SSRs) are wide range extensive length polymorphisms used in selection of marker-assisted, genetic diversity print-finger, genetic mapping or breeding applications. SSRs are usually made of 1 to 6 repeat

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nucleotides, which are composed in tandem and are widely distributed in the coding regions and non-coding regions of eukaryotic genes **[5]**. Screening of EST-SSRs from the cDNA library is simple, inexpensive, and sequence-consistent, hence it is widely used in various plants and fungi such as rice, coffee, beans, rubber tree **[6]**.

Methodology:

Fungi materials:

A cinnamemea AC001 (Genbank NO: KM925002) was donated by Taiwan Shennong Fungus Biotechnology Co., Ltd. and stored in the National Engineering Research Center of JUNCAO Technology of Fujian Agriculture and Forestry University. The strain was inoculated into a PDA plate and placed at a constant temperature of 28°C, then transferred to a liquid medium **[7]**, shaken at 28°C, 120 rpm in the dark, and cultured for 14, 21, 28, and 35 days. The mycelium was uniformly mixed after 35 days and used to construct a full-length cDNA library.

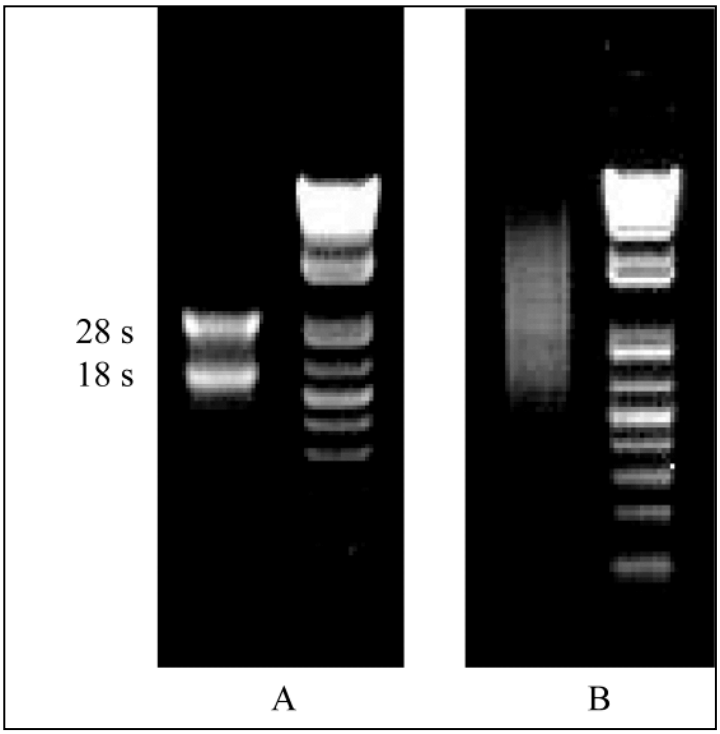

Figure 1: Electrophoresis of total RNA and mRNA of *A. cinnamomea* mycelium

Total RNA extraction and integrity assessment:

The total RNA of A. cinnamomea was extracted by TRIzol method **[8]**, and the total RNA integrity and quality analysis detected by electrophoresis on a 1% agarose gel and Nanodrop2000C (Thermo, USA). The mRNA was isolated by the FastTrack MAG Maxi mRNA Isolation Kit (Thermo, USA). cDNA synthesis was performed by the CloneMiner II cDNA Library Construction Kit (Thermo, USA).

Full-length cDNA library construction:

The cDNA library of A. cinnamomea was constructed by using the Gateway method[9]. Briefly, the cDNA was used as a template to synthesize the first strand and the second strand of cDNA. The cDNA obtained by transcription was linked to three different reading frames of the adaptor, and Homologous Recombination performed the recombinant method. The primary cDNA library was prepared by cloning into the pDONR222 vector. Then, the primary cDNA library mixed plasmid was extracted, and a yeast two-hybrid cDNA library (secondary library) was prepared by LR recombination into the yeast vector pGADT7-DEST. Library identification and determination. The primary and secondary libraries of the obtained cDNA were diluted in a ratio of 1:1000 and coated with a plate. After incubating for 10 h at 37°C, single colonies were observed and counted. The titer of the amplified library was calculated as follows: (number of plaques × dilution factor × 10^3 μL/mL)/(diluted phage plated μL). The dilution factor is 1 × 10^4. The inserted sequences were randomly selected for PCR amplification using pDONR222-F primers: 5'-TCCCAGTCACGACGTTGTAAAACGACGGCCAGTCTT-3', pDONR222-R primers: 5'-AGAGCTGCCAGGAAACAGCTATGAC CATGTAATACGACTC-3'. The total volume of PCR was 20 μL, containing 1 μL template, 10 μL 2×PCR Master Mix (Thermo, USA), 1 μL each primer and 7 μL ddH_2O. Cycling conditions were as follows: 94°C for 5 min, followed by 29 cycles of 95°C for 30 s, 57°C for 40 s and 72°C for 60 s, followed by 72°C for 5 min.

Bioinformatic analysis:

The clone transformants were randomly selected and sequenced by Bio-Sun Biotechnology Co., Ltd (Fujian, China). The adapter sequences were trimmed by Vecscreen tool (www.ncbi.nlm.nih.gov/tools/vecscreen) of National Center for Biotechnology Information (NCBI) and the inaccurate bases, poly-A tails, low-quality sequences (<100 bp) and other fragments **[10]** by CAP3 software removed to finally obtain more accurate sequencing. The NCBI BLAST program was used to compare the spliced single copy sequence with the nucleic acid library (NT) and protein (NR) databases (www.ncbi.nlm.nih.gov/) to complete the homology alignment of BLASTX. The database results with E value <1×10^{-5} were generally regarded as a significant match **[11]**. NCBI's non-redundant protein database followed by the assignment of functionality via Gene Ontologies (GO) **[12]** using BLAST2GO (www.blast2go.com/) **[13]**. The GO analysis included functional classification of Molecular Function, Cellular Component, and Biological Process for EST sequence data by BLAST2GO software. Pathway assignments were mapped according to Kyoto Encyclopedia of Genes and Genomes (KEGG) database, while interaction protein and nucleic databases was conducted for biological functions by the STRING network (www. string-db.org/).

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EST-SSR analysis:
Screening of EST-SSR was performed using the GRAMENE SSR online tool (https://archive.gramene.org/db/markers/ssrtool) withthe standard length for searching for SSR including dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, hexanucleotide with a minimum number of repetitions of 7, 5, 4, 4, 3. The SSR frequency and length were statistically analyzed.

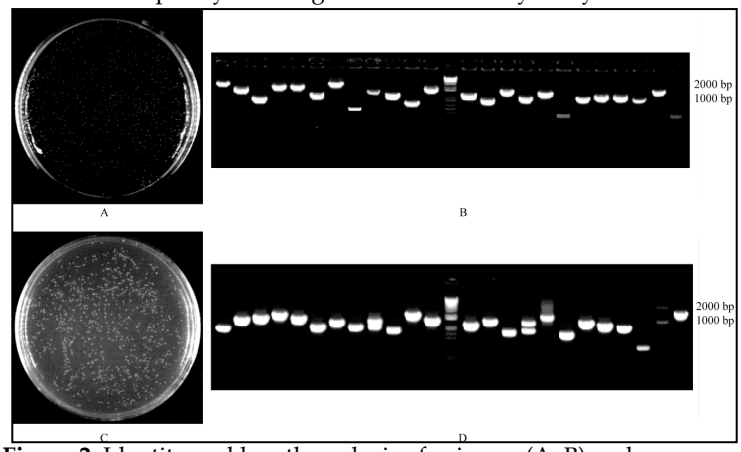

Figure 2: Identity and length analysis of primary (A, B) and secondary cDNA library (C, D)

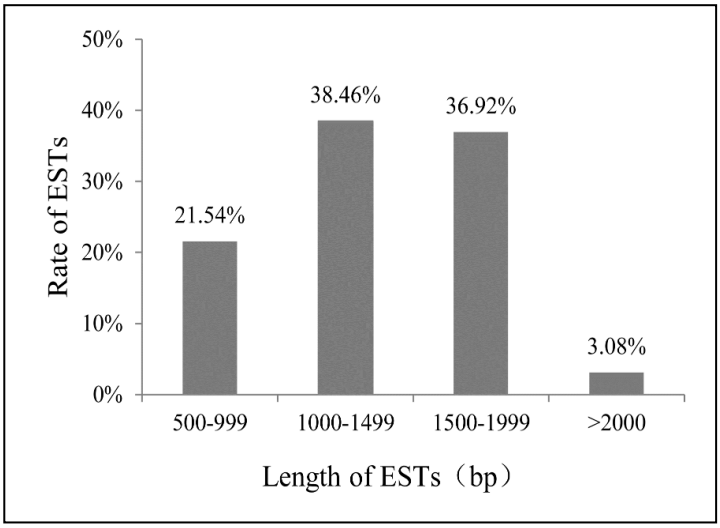

Figure 3: Distribution of ESTs lengths

Results & Discussion:

Total RNA assessment:
The total RNA extracted from the mycelium of A. cinnamomea was detected to be 963 ng/μL, the total amount was 481 μg, and the OD_{260}/OD_{280} was 2.14, which indicated that the total RNA was integrated and stable enough for cDNA library construction. The integrity was measured by 1% agarose gel electrophoresis as shown in **Figure 1A**, and mRNA was further isolated by total RNA isolation **(Figure 1B)**.

Construciton of cDNA library:
The full-length cDNA primary library was transformed into a plate dilution of 1:1000, the library titer was 5.2×10^6 CFU/mL, and the total number of clones was 1.0×10^7 CFU **(Figure 2A)**. The secondary library recombination rate was as high as 95%. The transformation plate dilution is 1:1000, the library titer is 6.1×10^6 CFU/mL, Total number of clones is 1.2×10^7 CFU **(Figure 2C)**. The clones were selected randomly and amplified by PCR using pDONR222-F and pDONR222-R primers. The PCR products were detected by 1% agarose gel electrophoresis and the recombinant rate was 95%. The insert size is 300-2000 bp, and the average length is 1000 bp **(Figures 2B and D)**, indicating that the library contains relatively long cDNAs that meet library capacity requirement. This method directly used cDNA as a template to synthesize cDNA double strands without any amplification process, and effectively kept the genetic information of full-length gene function. It also afforded an important resource for information about genes at the transcriptional level. The fragment is long enough to reflect the natural structure of the gene as much as possible, thus making it easier to obtain the complete sequence and functional information of the target gene in the library **[14]** and 95% of the recombinant cDNA library indicating the quality and providing a basis of the depth of the study.

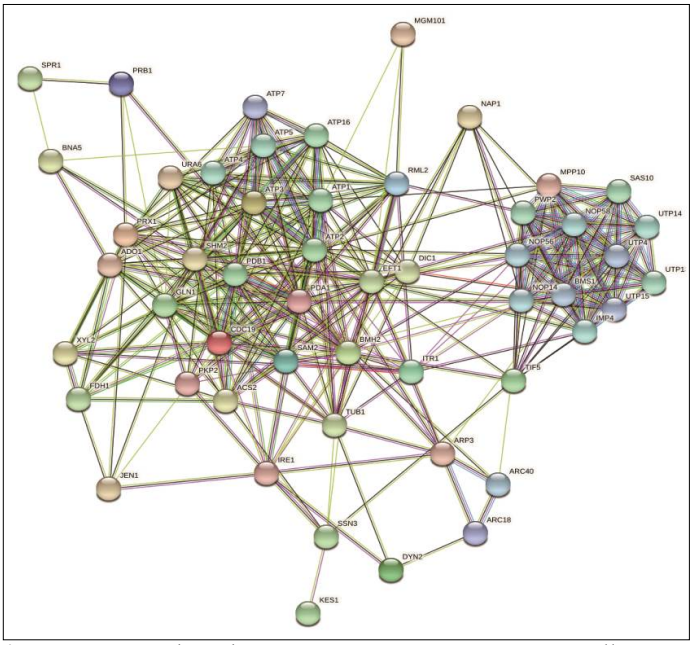

Figure 4: Network nodes represent proteins: experimentally determined (purple), gene neighborhood (green), gene fusions (red), gene co-occurrence (blue), co-expression (black)

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EST analysis:

Several clones were randomly picked from the cDNA library for sequencing, and a total of 65 valid single sequences (unigene) were obtained after removing the vector sequence and low-quality sequences (<100 bp). As shown in **Figure 3**, 14 ESTs length were 500-999 bp, 21 ESTs length were 1000-1499 bp, 24 ESTs length were 1500-1999 bp and 2 ESTs length were >2000 bp. Among them, the longest EST was 3474 bp, and the shortest EST was 573 bp. The CAP3 program was spliced to obtain a unique sequence consisting of one contigs and 64 single singlets. A total of 65 individual ESTs were analyzed and submitted in Genbank **(Table 1)**. The spliced sequence was subjected to homology alignment and gene function annotation with the NR database using NCBI BLASTX program. 65 single sequences except one (1.54%) had no significant homology; the remaining 64 (98.46%) had significant homologous sequences, including 46 known functional proteins and 18 unknown proteins. The homologous proteins were observed in Taiwanofungus camphoratus, Grifola frondosa, Wolfiporia cocos and fibroporia radiculosa etc. Obviously, 1 (Genbank NO.: MN205389) of the ESTs of the constructed library was noted to be novel and uncharacterized, and putatively expressed proteins with no homology matches of any public databases. Thus, its functional characterization and possible role in A. cinnamomea will be worthwhile to investigate.

Table 1: Comparison between ESTs with protein database of a cinnamomea mycelium cDNA liabary

NO.	Name	BLASTX protein & Accession NO.	Max Score	E-value	Identities	Genbank Accession NO.
1	AC2	NAD-dependent formate dehydrogenase [Gelatoporia subvermisporaB, EMD37341.1]	702	0	86.12%	MN205326
2	AC3	Mitochondrial carrier[Laetiporus sulphureus, KZT01943.1]	484	5e-168	84.75%	MN205327
3	AC4	1-Cys peroxiredoxin[Taiwanofungus camporatu, AAX59894.1]	462	7e-164	100%	MN205328
4	AC5	NAD (P)-binding protein[Wolfiporia cocos, PCH39423.1]	253	6e-92	66.84%	MN205329
5	AC9	Phosphatidylserine synthase 2[Wolfiporia cocos, PCH39820.1]	781	0	87.95%	MN205330
6	AC12	Carboxylic acid transporter [Wolfiporia cocos, PCH39927.1]	751	0	74.79%	MN205331
7	AC13	Predicted protein [Fibroporia radiculosa, XP_012178603.1]	103	3e-21	47.37%	MN205332
8	AC14	Alpha-ketoacid dehydrogenase kinase[Obba rivulosa, OCH94093.1]	646	0	77.18%	MN205333
9	AC15	Acetyl-coenzyme a synthetase[Laetiporus sulphureus, KZT08091.1]	1205	0	90.94%	MN205334
10	AC17	Dipeptidyl-peptidase 5 [Sparassis crispa, XP_027615982.1]	441	0	85.04%	MN205335
11	AC18	Zn-dependent exopeptidase[Wolfiporia cocos, PCH40884.1]	590	0	75.79	MN205336
12	AC21	Methionine adenosyltransferase[Obba rivulosa, OCH88795.1]	722	0	93.62%	MN205337
13	AC22	E3 ubiquitin-protein ligase UHRF1 [Grifola frondosa, OBZ72511.1]	201	3e-56	53.37%	MN205338
14	AC23	Sorbitol dehydrogenase[Grifola frondosa, OBZ67832.1]	709	0	91.01%	MN205339
15	AC27	Hypothetical protein LAESUDRAFT_672451[Laetiporus sulphureus, KZT10884.1]	372	9e-124	67.48%	MN205340
16	AC28	Hypothetical protein LAESUDRAFT [Laetiporus sulphureus, KZT03671.1]	218	3e-68	68.92%	MN205341
17	AC29	Cytochrome P450 monooxygenase[Taiwanofungus camphoratus, AEB40226.1]	1075	0	94.93%	MN205342
18	AC30	Hypothetical protein SCP_0904850[Sparassis crispa, XP_027617519.1]	114	2e-26	43.46%	MN205343
19	AC31	UMP-CMP kinase[Obba rivulosal, OCH95225.1]	329	1e-109	71.03%	MN205344
20	AC33	Vacuolar amino acid transporter 2[Sparassis crispa, XP_027611999.1]	356	1e-116	87.50%	MN205345
21	AC34	U3 small nucleolar RNA-associated protein MPP10 [Grifola frondosa, OBZ79155.1]	564	0	66.34%	MN205346
22	AC35	Hypothetical protein OBBRIDRAFT [Obba rivulosa, OCH84396.1]	181	7e-47	62.22%	MN205347
23	AC36	DUF1909-domain-containing protein [Polypororus brumalis, RDX56099.1]	131	8e-35	85.14%	MN205348
24	AC37	NAP-domain-containing protein [Laetiporus sulphureus, KZT00903.1]	290	2e-110	80.93%	MN205349
25	AC38	Predicated protein [Fibroporia radiculosa, XP_012185683.1]	339	3e-112	64.48%	MN205350
26	AC39	Hypothetical protein POSPLADRAFT [Postia placenta, XP_024336598.1]	545	0	74.93%	MN205351
27	AC40	Hypothetical protein HYDPIDRAFT_29335[Hydnomerulius pinastri, KIJ63539.1]	89	1e-17	70.31%	MN205352
28	AC41	Kynureninase [Wolfiporia cocos, PCH38103.1]	701	0	77.85%	MN205353
29	AC43	cytochrome P450 [Laetiporus sulphureus, KZT01907.1]	624	0	62.18%	MN205354
30	AC46	Mitochondrial genome maintenance MGM101 [Polyporus brumalis, RDX53803.1]	282	1e-91	90.41%	MN205355
31	AC49	NADH dehydrogenase [Wolfiporia cocos, PCH40004.1]	595	0	78.36%	MN205356
32	AC52	Predicated protein [Fibroporia radiculosa, XP_012181539.1]	558	0	83.2%	MN205357
33	AC53	Indoleamine 2,3-dioxygenase [Wolfiporia cocos, PCH39352.1]	597	0	72.12%	MN205358
34	AC55	pyruvate kinase[Tametes coccinea, OSD03555.1]	938	0	87.55%	MN205359
35	AC57	Predicated protein [Fibroporia radiculosa, XP_012185683.1]	282	2e-91	64.66	MN205360
36	AC58	Subtilisin like protease [Sparassis crispa, XP_027611768.1]	760	0	88%	MN205361
37	AC59	Tubulin alpha-1a chain [Grifola frondosa, OBZ76459.1]	795	0	98.07%	MN205362
38	AC61	14-3-3-1 protein [Daedalea quercina, KZT71517.1]	508	3e-180	98.02	MN205363
39	AC63	Dynein light chain, flagellar outer arm [Hypsizygus marmoreus, RDB23343.1]	193	4e-60	91.89	MN205364
40	AC73	ATP synthase F1 gamma [Wolfiporia cocos, PCH36766.1]	543	0	88.18%	MN205365
41	AC75	Kinase-like protein [Wolfiporia cocos, PCH43934.1]	664	0	81.00%	MN205366
42	AC76	Alpha/beta-hydrolase [Wolfiporia cocos, PCH40344.1]	600	0	74.35%	MN205367
43	AC77	Glycoside hydrolase family 5 protein [Postia placenta, XP_024338264.1]	323	3e-98	67.5%	MN205368
44	AC78	PpCYP014 mRNA Cytochrome P450 [Postia placenta, BAK09377.1]	590	0	62.2%	MN205369
45	AC79	Hypothetical protein MPER_04035[Moniliophthora perniciosa, EEB96775.1]	73.6	9e-13	85.17%	MN205370
46	AC83	Predicated protein [Fibroporia radiculosa, XP_012180973.1]	607	0	70.13%	MN205371
47	AC84	Calreticulin domain containing protein [Daedalea quercina, KZT72961.1]	794	0	84.07%	MN205372

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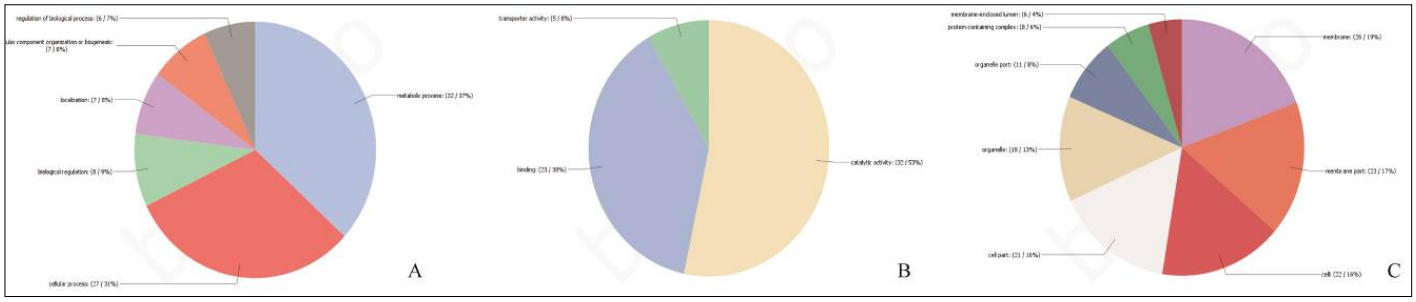
48	AC86	Dicarboxylic acid transporter [Laetiporus sulphureus, KZT06709.1]	525	0	89.61%	MN205373
49	AC88	Putative monooxygenase [Serpula lacrymans, XP_007324373.1]	598	0	61.07%	MN205374
50	AC90	Myo-inositol transporter 1[Sparassis crispa, XP_027614396.1]	707	0	82.95%	MN205375
51	AC91	Hypothetical protein SCP 1001120[Sparassis crispa, XP_027617783.1]	707	0	71.19%	MN205376
52	AC93	Actin-related protein [Sparassis crispa, XP_027618707.1]	777	0	95.13%	MN205377
53	AC95	Subtilisin-like protease [Sparassis crispa, XP_027611768.1]	730	0	74.9%	MN205378
54	AC96	Glutamate-ammonia liqase [Stecchemrinum ochraceum, TCD69661.1]	697	0	94.02%	MN205379
55	AC98	Hypothetical protein pospladraft_1071673[Postia placenta, XP_024334193.1]	802	0	85.78%	MN205380
56	AC99	Adenosine kinase [Sparassis crispa, XP_027608215.1]	552	0	83.97%	MN205381
57	AC100	Hypothetical protein PHMEG_00017208[Phytophthora meqakarya, OWZ10005.1]	77.8	6e-15	82.98%	MN205382
58	AC101	Oxysterol-binding protein [Wolfiporia cocos, PCH43009.1	680	0	84.03%	MN205383
59	AC102	Eukaryotic translation initiation factor 5[Laetiporus sulphureus, KZT08930.1]	591	0	87.76%	MN205384
60	AC104	Hypothetical protein SCP_1302980[Sparassis crispa, XP_027619395.1]	546	0	75.50%	MN205385
61	AC106	Alpha/beta-hydrolase [Wolfiporia cocos, PCH37718.1]	505	6e-172	68.29%	MN205386
62	AC107	Hypothetical protein BN946_scf184934.g8scf184934 [Trametes cinnabarina, CDO75793.1]	182	1e-55	67.61%	MN205387
63	AC108	Serine hydroxymethyltransferase 3[Grifola frondosa, XP_027620033.1]	915	0	90.59%	MN205388
64	AC109	NONE				MN205389
65	AC110	Predicted protein [Fibroporia radiculosa, XP_012179294.1]	669	0	83.67%	MN205390

Table 2: KEGG Pathway analysis of ESTs

KEGG ID	Term description	Gene count	False discovery rate	Matching proteins
Sce03008	Ribosome biogenesis in eukaryotes	10	1.80E-08	BMS1, IMP4, MPP10, NOP56, NOP58, PWP2, UTP13, UTP14, UTP15, UTP4
Sce01100	Metabolic pathways	19	2.07E-05	ACS2, ADO1, ATP1, ATP16, ATP2, ATP3, ATP4, ATP5, ATP7, BNA5, CDC19, FDH1, GLN1, PDA1, PDB1, SAM2, SHM2, URA6, XYL2
Sce00190	Oxidative phosphorylation	7	2.39E-05	ATP1, ATP16, ATP2, ATP3, ATP4, ATP5, ATP7
Sce01200	Carbon metabolism	6	0.0023	ACS2, CDC19, FDH1, PDA1, PDB1, SHM2
Sce00620	Pyruvate metabolism	4	0.0029	ACS2, CDC19, PDA1, PDB1
Sce00010	Glycolysis / gluconeogenesis	4	0.0058	ACS2, CDC19, PDA1, PDB1
Sce00680	Methane metabolism	3	0.0058	ACS2, FDH1, SHM2
Sce00630	Glyoxylate and dicarboxylate metabolism	3	0.0068	FDH1, GLN1, SHM2



Supplementary Figure 1: Classification of identified genes based on the relevant biological process (A), Molecular Fucction (B) and celluar components (C).

Table 3: Repeat motifs in EST-SSRs of *A. cinnamomea*

Items	NO.	SSR
Di-nucleotide	12	AG, AC, AT, TC, TA, TG, CT, CA, CG, GT, GA, GC
Tri-nucleotide	41	AAG, ACA, ATC, AGC, AAG, TCA, TTC, TCC, TCG, TTG, TGC, TGG, CGG, CTG, CTC, CGC, CAC, CAT, CCT, CAG, CAA, GGC, GTC, GTT, GCC, GGT, GAT, GCG, GCT, GAC, GTG, TTA, GAG, GCC, GAC, CGT, GAA, CGA, GGA, GAT, GGA
Tetra-nucleotide	1	ATAC
hexa-nucleotide	0	--
hept-anucleotide	1	CTGGGG,
Others	1	CGTGAGG

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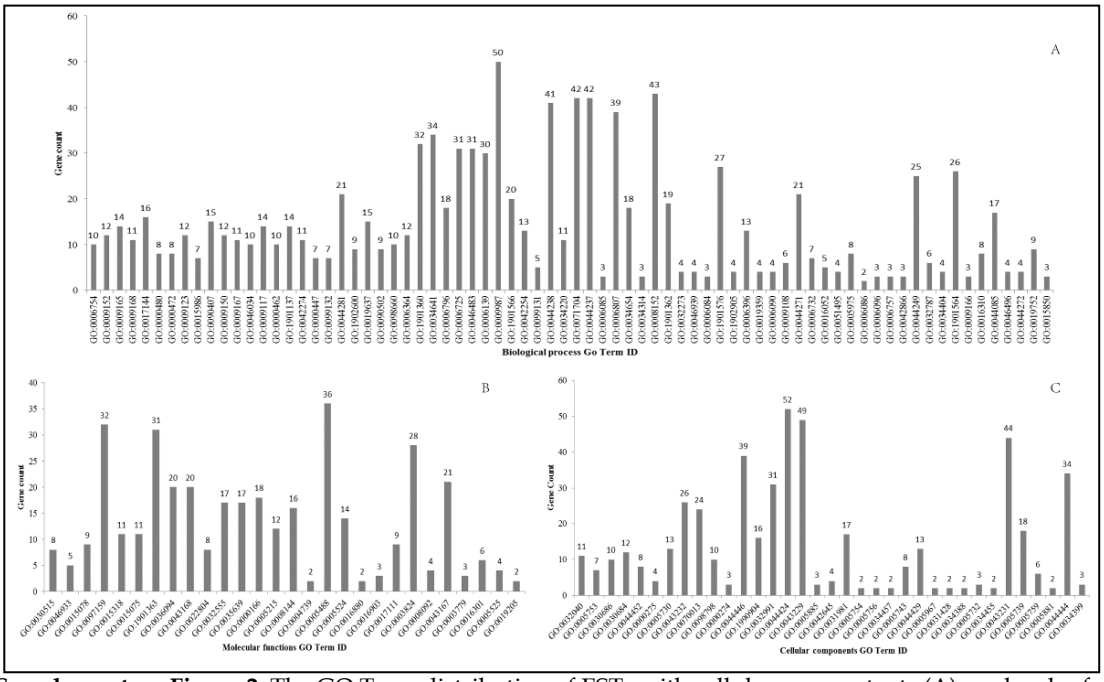

Supplementary Figure 2: The GO Term distribution of ESTs with cellular compontents (A), molecular function (B) and biological processes (C).

GO annotation analysis:

The single sequence of functional annotations was classified into functional categories in BLAST2GO software. A total of 227 GO terms were obtained, with an average of 6.837, divided into 34.36% biological processes, 27.75% cellular components, and 37.88% molecular functions, with the distribution of the ESTs **(Suplementary Figure 1)**. According to the biological process, the component contained metabolic process (37%), cellular process (31%), biological regulation (9%), localization (8%), cellular component organization or biogenesis (8%) and regulation of biological process (7%), as shown in **Supplementary Figure 1A**. Regarding molecular function, 53% ESTs were associated with catalytic activity, 38% ESTs were associated with binding and only 8% ESTs were associated with transporter activity **(Supplementary Figure 1B)**. The components of biological cellular process associated with the ESTs included membrane (19%), membrane part (17%), cell (16%), cell part (16%), organelle (13%), organelle part (8%), protein-containing complex (6%) and membrane-enclosed lumen (4%) **(Supplementary Figure 1C)**. Supplementary **Figure 2A and 3A** shows the enrichment of gene functions. There are 5 GO terms in biological process that have highly enrichment value including cellular process (GO:0009987), phosphorylation (GO:0016310), cellular metabolic (GO:0044237), metabolic process (GO:0008152), oxidation-reduction process (GO:0055114), with 27, 6, 21, 32, 10 background genes respectively. According to molecular function GO term, there is catalytic activity (GO: 0003824) and oxidoreductase activity (GO:0016491) significantly enriched with 32 and 10 background genes **(Supplementary Figure 2B and 3B)**. In cellular component GO term, cell nodescore (GO: 0005623), membrane nodescore (GO:0016020), intracellular part (GO: 0044424), integral component (GO:0016021), with 22, 26, 21 and 23 backgroud genes, respectively **(Supplementary Figure 2C and 3C)**.

Table 4: Di-nucleotide and tri-nucleotide repeat motifs in ESTs of *A. cinnamomea*

Items	SSR	NO.	Rate of ESTs
Di-nucleotide	GC	40	14.76%
	CG	36	13.28%
	CT	26	9.59%
	TC	25	9.22%
	CA	18	6.64%
	TG	16	5.90%
	AT	13	4.80%
	GA	11	4.06%
	AC	10	3.69%
	GT	8	2.95%
	AG	6	2.21%
	TA	4	1.48%
Tri-nucleotide	AGG	3	1.11%
	GTC	3	1.11%
	GAA	3	1.11%
	CAG	2	0.74%
	CGT	2	0.74%
	GCG	2	0.74%
	GAT	2	0.74%
	GTG	2	0.74%
	TCC	2	0.74%
	TCG	2	0.74%
	TGC	2	0.74%
	TTG	2	0.74%

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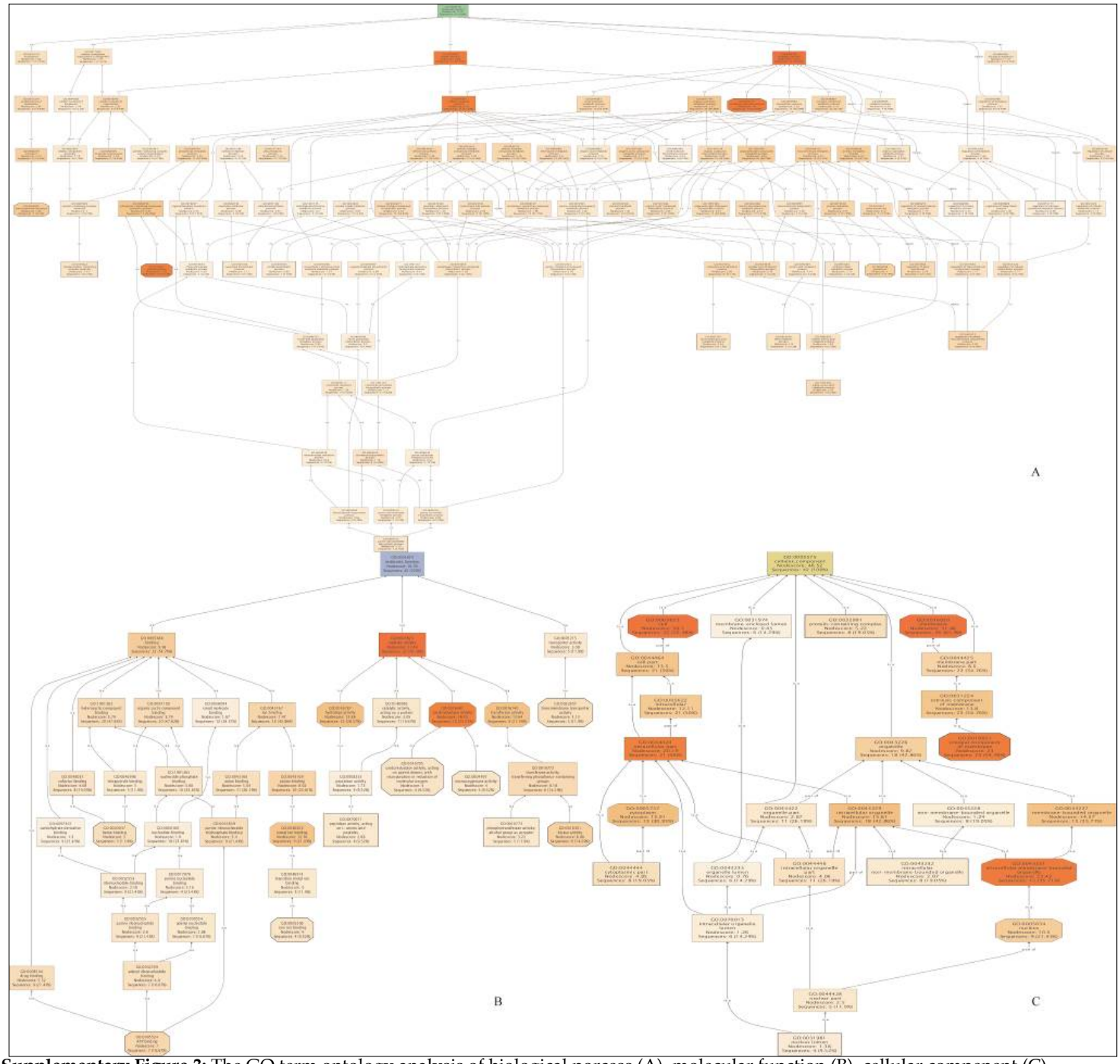

Supplementary Figure 3: The GO term ontology analysis of biological porcess (A), molecular function (B), cellular compenent (C)

KEGG pathway and Interaction protein analysis:

KEGG is an approach to link genomic data with higher order functional sequence by computerizing current information on cellular processes and by standardizing gene annotations. It provides biochemical pathways for the annotations species in which the genome have been discovered. According to **Table 2**, a

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total of 56 matching proteins were revealed to be involved in 8 KEGG pathways. 19 of 65 ESTs were annotated to the metabolic pathways (sce01100), which are the most represented pathways. While, the ribosome biogenesis in eukaryotes and oxidative phosphorylation are the second and third most represented pathways. In addition, carbon metabolism, pyruvate metabolism, glycolysis gluconeogenesis, methane metabolism and glycosylate and dicarboxylate metabolism were also represented. The interaction among the 65 ESTs were analysed by STRING database with the genome of Sachharomyces cerevisiae. The result showed a functional association network determined with 53 codes, 346 edges and PPI enrichment p-value of 0.00131 **(Supplementary Table 1 see linked excel file)**. The 65 ESTs with the low confidence (0.150) minimum required interaction score is no more than 20 interactors. The predicted potential regulators with no clustering are shown in **Figure 4**. The STRING results revealed protein ATP2, PDB1, ATP1, ATP16, SAS10, PWP2, UTP13, ATP5, ATP4, UTP14, NOP58, NOP14, NOP56, ARC40, BMS1, UTP4, UTP15, ATP7, ARC18 were the functional partners. As Supplementary Figure 1 shows, there are a total of 11 GO terms which have high enrichment value including cellular process, phosphorylation, cellular metabolic, metabolic process, oxidation-reduction process, catalytic activity and oxidoreductase acitivity, cell nodescore, membrane nodescore, intracellular part, integral component of the A. cinnamomea cDNA library. In the present study, the STRING for *A. cinnamomea* is primarily based on the *Sachharomyces cerevisiae* genome. We sequenced among the matches, 29 genes which encoded enzymes highly involved in Ribosome biogenesis in eukaryotes, oxidative phosphorylation, carbon metabolism, glycolysis/ gluconeogenesis, glyoxylate and dicarboxylate metabolism, pyruvate metabolism and methane metabolism **(Table 2)**.

EST-SSR analysis:

A total of 271 SSRs were isolated from 63 ESTs of A.cinnamomea with an SSR occurrence frequency of 96.82%. Among the EST-SSRs, there are 7 ESTs with 1 SSR, 12 ESTs with 2 SSRs, and 44 ESTs with 3 or more. The tri-nucleotides results shown in **Table 3** accounts for the largest proportion of all SSRs. The di-nucleotide and tri-nucleotide repeat SSRs in the EST accounted for 95.31% of the total repeats, and the number of dinucleotide repeat motifs GC, CG, CT, and TC appeared more frequently with repeats of 14.76%, 13.28%, 9.59%, and 9.22%, respectively **(Table 4)**. However, AG, GT, and TA were less frequent motifs accounting for less than 5%. In the tri-nucleotide repeat motif, AGG, GAA and GTC appear slightly higher, accounting for 3.33%, while CAC, CGC, AGC and ATC only occur once. The frequencies of tetra-nucleotides, hexa-nucleotides and hepta-nucleotides are all very low. However, di-nucleotide SSRs (18.75%) and tri-nucleotide SSRs (76.56%) presented higher polymorphic proportions than tetra- nucleotide in A. cinnamomea, which suggested that the SSRs which occurred within untranslated region were more polymorphic than those in exon regions.

Conclusion:

In this study, we document some insights from the cDNA and EST analysis of *Antrodia cinnamomea* for further data mining. This is helpful in the genome level understanding of *Antrodia cinnamomea* for applciation in biomedicine.

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Conflicts of interest: All authors declare no conflict of interest.

References:

[1] [1] Geethangili M & Tzeng YM. Evidence-Based Complementary and Alternative Medicine. 2011. [PMID: 19687189].
[2] [2] Lu M-Y J *et al.* Proceedings of the National Academy of Sciences. 2014 111(44): E4743-E4752 [PMID: 2533675].
[3] [3] Mao X-G, et al. Hereditas. 2006 28(7): 865-873 [PMID: 16825176].
[4] [4] Weiste C, et al. Plant Journal. 2010 52(2): 382-390 [PMID: 17672839].
[5] [5] Gupta P K&Varshney R. Euphytica. 2000 113(3): 163-185.
[6] [6] Ellis J&Burke J. Heredity. 2007 99(2): 125 [PMID: 17519965].
[7] [7] Li J, et al. Souththwest China Journal of Agricultural Sciences. 2015 28(6): 2649-2654.
[8] [8] Lee J T Y, et al. Journal of Biomaterials and Nanobiotechnology. 2011 2(01): 41.
[9] [9] Cao S, et al. BMC biotechnology. 2011 11(1): 53 [PMID: 21595971].
[10] [10] Huang X&Madan A. Genome research. 1999 **9**:868. [PMID: 10508846].
[11] [11] Joshi A G&Pathak A R. Plant and Human Health, Volume 2. 2019 Springer. 207. [PMID: 19840835].
[12] [12] Ashburner M, et al. Nature genetics. 2000 25(1): 25 [PMID: 10802651].
[13] [13] Conesa A, et al. Bioinformatics. 2005 21(18): 3674-3676 [PMID: 16081474].
[14] [14] Clarke L&Carbon J. Cell. 1976 9(1): 91-99 [PMID: 788919].

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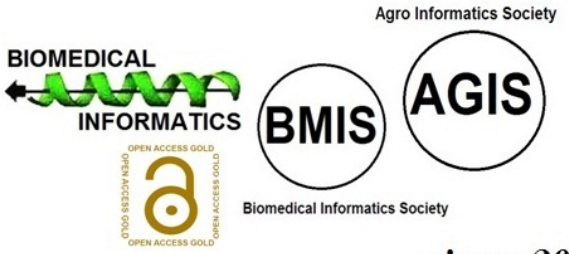

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