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Original paper

GC/MS-based metabolic profiling reveals important metabolic pathways in microsporidia, *Nosema antheraeae*

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Abstract

The microsporidian, *Nosema antheraeae* can infect Chinese oak silkworm, Antheraea pernyi, and cause pebrine disease. This disease could greatly reduced the income from tussah industry. For seeking a way to overcome this disease, more and more researchers were worked for exploring the infection mechanism of the spores. Here, in order to identify the metabolites of *N. antheraeae* for analyzing key metabolic pathways, Gas chromatography-mass spectrometry (GC/MS)-based metabolome was performed.

1975 metabolic characteristic peaks were assignment and finally fifty-two metabolites were identified, including amino acid, fatty acid, sugars, etc. Database searches showed that the monoisotopic mass of the obtained metabolites ranged from 57 to 539 Da. Metabolic pathway enrichment analysis revealed that many basic metabolism processes, including protein biosynthesis, amino acid metabolism, pentose phosphate pathway, etc were found. These results, for the first time, provided a tool to explore the metabolic profiling of the microspridia and may provide a new horizon for seaking pathogenic molecules during infection of spores.

Keywords Metabolites; pathway analysis; *Antheraea pernyi*; pathway enrichment; energy metabolism.

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Introduction

The Chinese oak silkworm, *Antheraea pernyi*, which belongs to Lepidoptera: Saturniidae, is an important economic insect in North China for Tussar silk production, insect food and medicinal materials. In China, annual output of tussah cocoon is high to 7×10^4 t and approximately exports accounts for 90% of the world total of wild silk. Tussah production has become an important pillar industry of rural economy in most sericultural areas.

As a main pathogen, which can systematically infect *Antheraea pernyi*, *Nosema antheraeae* (*Na*), could lead to serious pebrine disease (XU & al [1]). This disease has greatly reduced the income from tussah industry and hindered the development of sericulture in China (WANG & al [2]). For seeking a way to overcome this disease, more and more studies were reported, which mostly focused on the structure of *N. antheraeae* spores, function of identified proteins and mechanism of the infection in recent years (WANG & al [3], TANG & al [4], GONG & al [5]). But no efficient method was obtained to prevent the pestilence, thus it was urgent to exploit a new research field. With the development of science and technology, metabolomics had been developed and it had become a new tool to analyze all the low molecular weight (less than 1000) metabolites in a cell at a certain time. It becomes a major component of systems biology. Up to now, it is a powerful tool not only for metabolite identification (VANYUSHKINA & al [6]) , but also for the areas closely associated with human health and nursing, such as disease diagnosis (HUANG & al [7]), nutrition and food science, environmental science and so on. Most notably, metabolomics could not only play an important role in exploring the mechanisms of biological response (Barding & al [8]), but also provide clues to mechanistic research (CHEN & al [9]). At present, a variety of analytical techniques were applied to metabolomics research. For the higher resolution and detection sensitivity, gas chromatography-mass spectrometry (GC-MS) was widely used for metabolite identification in the area of animal biology, plant physiology, etc. (CHEN & al [9], SALANȚĂ & al [10]).

So far, more and more studies were reported to explore the functional molecules and infection mechanism of microsporida (YANG & al [11]). Host responses for seaking clues to reveal the immunization strategies or to control the spread of insect microsporidiosis, were also becoming a research hotspot (MA & al [12]). However, nearly all of the reported works were achieved using approaches describing changes on the genome level, proteome level or transcriptome level. Although metabolomics methods are efficient to reveal the correlation of genotypes and phenotypes and to understand the essential metabolic processes during the parasitic life, no studies analyzed the metabolome of microsporidia was reported. Here, this work aimed to identify as many intracellular metabolites as possible in microsporidia, *Nosema antheraeae*, for obtaining the metabolic profiling for revealing key metabolic pathways in mature spores.

Materials and Methods

Experimental spores and treatments

Nosema antheraeae was obtained from infected pupa of Chinese oak silkworm (*Antheraea pernyi*). Briefly, infected pupa was grinded in a mortar and suspended in sterile water. Spore purification was on the basis of the method reported for purifying microsporidia (*Nosema bombycis*) from silkworm (*Bombyx mori*) (MA & al [12]). Then, spores were purified with a percoll gradient centrifugation (10, 25, 50, 75 and 90%, v/v) under aseptic conditions. The pellets of mature spores were rinsed twice with sterilized double distilled water and stored with antibiotics (100 μg/ml streptomycin, 100 U/ml penicillin) for later use (MA & al [12]).

Sample treatment for Gas chromatography-mass spectrometry (GC-MS) analysis

An improved sample preparation was refered based on the method for sample preparation in silkworm (CHEN & al [9]). In order to get rid of proteins, the *Na* samples were diluted with acetonitrile (4×10^9 spores: 400 μL), these mixtures were violently vortexed for 2 min with glass beads and three repeats were needed. Then placed on ice for 1 h, after that, all the mixtures were centrifuged to deproteinize under 4℃ at 13,000 *g* for 15 min. The collected supernatants (320 μL) were transferred and lyophilized. Then, dissolving the lyophilized residues using 100 μL of methoxyamine solution (20 mg/mL in pyridine) and followed by water bath at 37℃ for 60 min and ultrasound for 20 min, successively. Nextly, 80 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was used for silylation under the same conditions for 60 min. To stop the reflection, 20 μL of N-heptane was employed and followed by centrifugation under 4°C at 14,000 *g* for 25 min. Finally, 120 μL of supernatants were obtained for GC-MS analysis. It is worth mentioning that instrument parameters for GC-MS analysis was referred to the reported work (CHEN & al [9]).

GC-MS data processing and analysis

First, CDF data format files were generated from the raw data files using Agilent ChemStation, and then the m/z features was matched using XCMS Online. Finally, the NIST 2011 (version 2.0) library and standard samples were used to analyze their mass spectra (LI & al [13]).

Results

MS spectra acquisition

Spores were purified from infected pupa of *Antheraea pernyi* using percoll gradient centrifugation. Glass beads were used to destroy cell walls to release metabolites and samples were harvested using acetonitrile for metabolite identification. Metabolite detection was accomplished by GC-MS. In order to obtain the most reliable data, the experimental parameters and the mass spectrometric peak recording, were optimised by standards.

Total ion current (TIC) was shown in Fig. S1. The NIST 2011 library was used for metabolites identification. There were three biological-independent repeats for metabolite detection. In order to reduce the repetitive error, two technological repeats were performed for each biological repeat. The two step chemical derivatization method was used to inhibit the multiple peaks of the reducing sugar (PASIKANTI & al [14]). In this study, 1975 metabolic characteristic peaks were assignment and finally fifty-two metabolites were identified.

Metabolite content of *Nosema antheraeae*

After identification using the NIST 2011 library, twenty-six metabolites were unknown and fifty-two metabolites were obtained (Table 1). According to certain features of the identified metabolites, the classification was as follows: carbohydrates, lipids, amino acid and others (shown in Fig. 1). In the data, fourteen amino acids were identified and took almost 27% of the identified metabolites, including Glycine, Alanine, Lysine etc. Fatty acid and carbohydrates were also identified which could provide energy and substrates during the process of *N. antheraeae* life cycle. Remarkably, trehalose, as an important production of trehalose synthetic pathway, was also found here and this suggested that similar to other microsoridian, such as *Nosema bombycis*, *Encephalitozoon cuniculi* and so on, trehalose metabolic pathway was also as the primarily-dependent pathway in *N. antheraeae* to produce glucose for causing gemination (UNDEEN & VANDERMEER [15]) and supporting energy (VIVARES & al [16]).

Database searches showed that the monoisotopic mass of the obtained metabolites ranged from 57 to 539 Da (Table 1). Based on monoisotopic mass, all the obtained metabolites were divided into five groups (Fig. 2). Notablely, the proportion of metabolites with monoisotopic mass ranging from 100 to 400 Da was as high as 89.8%.

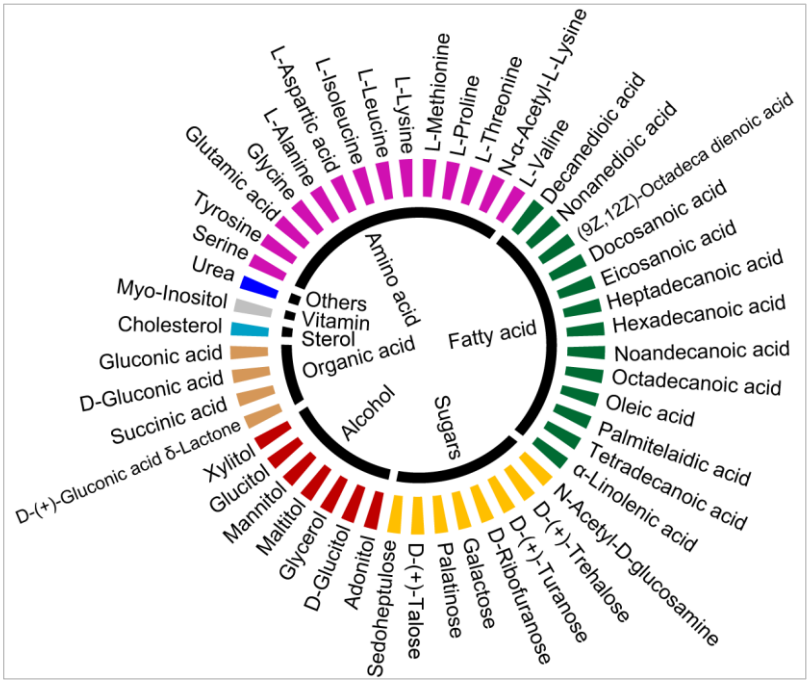

Figure 1. Analysis of metabolites of microsporidia, *Nosema antheraeae*, identified by GC-MS technique. List and classification of identified metabolites

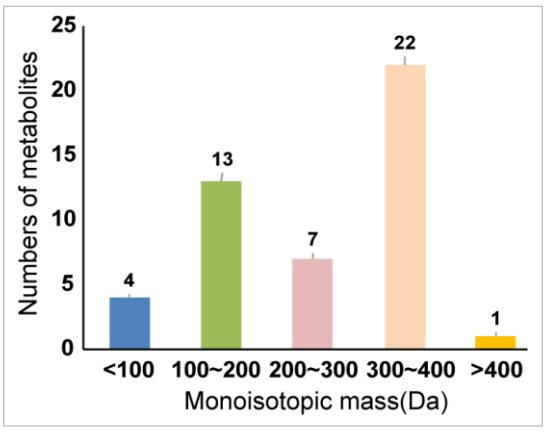

Figure 2. Monoisotopic mass distribution of the identified metabolites

Table 1. Identification of metabolites and their pathways of Microsporidia, *Nosema antheraeae*, by GC-MS technique

Metabolite	RT	HMDB	KEGG
Amino acid			
Glutamic acid	28.02	HMDB00148	C00025
Glycine	18.61	HMDB00123	C00037
L-Alanine	12.19	HMDB00161	C00041
L-Aspartic acid	22.26	HMDB00191	C00049
L-Isoleucine	14.34	HMDB00172	C00407
L-Leucine	17.63	HMDB00687	C00123
L-Lysine	34.46	HMDB00182	C00047
L-Methionine	21.64	HMDB00696	C00073
L-Proline	25.16	HMDB00162	C00148
L-Threonine	18.28	HMDB00167	C00188
N-α-Acetyl-L-Lysine	32.83	HMDB03759	C03681
L-Valine	11.51	HMDB00883	C00183
Serine	17.09	HMDB00187	C00065
Tyrosine	33.48	HMDB00158	C00082
Alcohol			
Adonitol	30.71	HMDB00508	C00474
D-Glucitol	42.95	HMDB00247	C00794
Glycerol	17.92	HMDB00131	C00116
Maltitol	53.07	HMDB02928	G00275
Mannitol	44.2	HMDB00765	C00392
Glucitol	35.32	HMDB00247	C00794
Xylitol	30.62	HMDB02917	C00379
Fatty acid			
(9Z,12Z)-Octadecadienoic acid	40.06	HMDB05047	C01595
Docosanoic acid	48.18	HMDB00944	C08281
Eicosanoic acid	44.6	HMDB02212	C06425
Heptadecanoic acid	38.71	HMDB02259	-
Hexadecanoic acid	36.71	HMDB00220	C00249
Nonadecanoic acid	42.68	HMDB00772	C16535
Octadecanoic acid	40.73	HMDB00827	C01530
Oleic acid	40.18	HMDB00207	C00712
Palmitelaidic acid	36.25	HMDB12328	-
Tetradecanoic acid	32.6	HMDB00806	C06424
α-Linolenic acid	41.93	HMDB01388	C06427
Decanedioic acid	22.34	HMDB00792	C02678
Nonanedioic acid	20.7	HMDB00784	C08261
Organic acid			
Succinic acid	18.86	HMDB00254	C00042
D-(+)-Gluconic acid δ-lactone	33.98	HMDB01127	-
D-Gluconic acid	36.59	HMDB00625	C00257
Gluconic acid	34.2	HMDB00150	C00257
Sterol			
Cholesterol	55.64	HMDB00067	C00187
Sugars			
D-(+)-Talose	34.42	-	C06467
D-(+)-Trehalose	51.03	HMDB00975	C01083
D-(+)-Turanose	52.68	HMDB11740	C19636
D-Ribofuranose	51.77	-	C16639
Galactose	37.9	HMDB00143	C00124
N-Acetyl-D-glucosamine	38.19	HMDB00215	C00140
Palatinose	53.58	-	C01742
Sedoheptulose	38.92	HMDB03219	C00447
Vitamin			
Myo-Inositol	38.3	HMDB00211	C00137
Others			
Urea	16.65	HMDB00294	C00086

* The ID of HMDB and KEGG were listed in the 3rd and 4th column.

Pathway Analysis

Pathway analysis allows to locate metabolites on known biochemical pathways, which provides clues to explore their possible genetic roles. Pathway analysis tools can play a role in finding out biological functions of metabolites in biological systems. Here, MetPA program was employed to perform metabolic pathway enrichment analysis of identified metabolites, result was shown in Fig. 3. The results showed that total forty-two metabolic pathways were involved. These data suggested that a wide range of metabolic pathways were worked and basic metabolism processes, such as protein biosynthesis, galactose metabolism, ammonia recycling and so on, were maintain at relatively high levels. Metabolic pathways related to energy metabolism, including citric acid cycle (TCA), pentose phosphate pathway and mitochondrial electron transport chain were also found here. Insulin signalling pathway, which can play a key role in regulation of gene transcription, protein synthesis, cell growth and gene expression, was also existed with a low metabolic abundance.

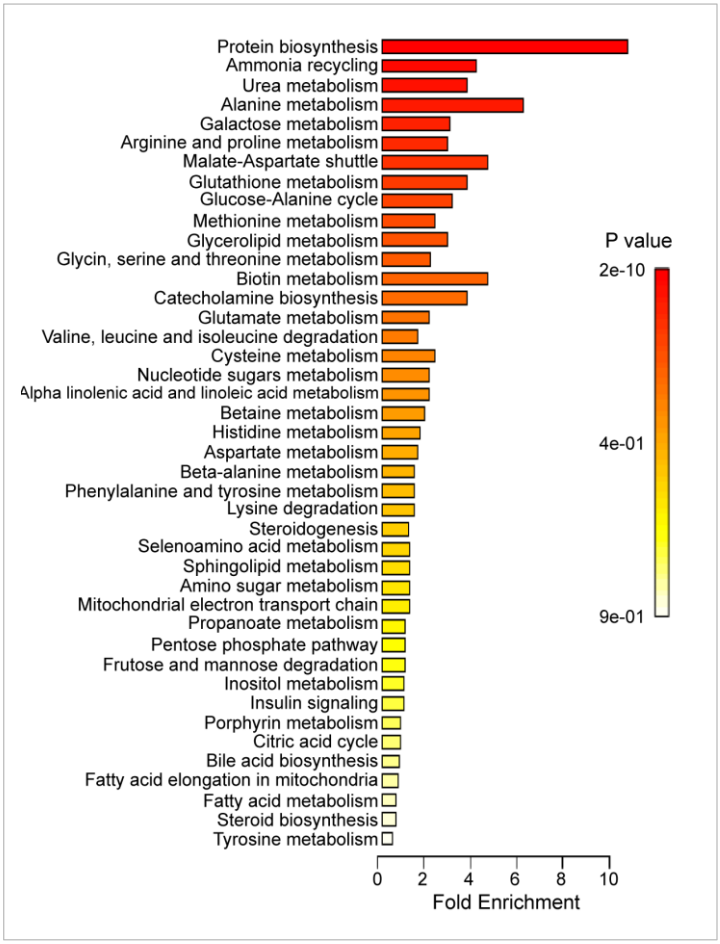

Figure 3. Metabolic pathway enrichment analysis result of *N. antheraeae* metabolites using MetPA program

Discussion

Microsporidia can infect a variety of hosts, reductive evolutions on genome and metabolome levels were verified to exist. *Enterocytozoon bieneusi* was reported missing core carbon metabolism, including pentose phosphate and trehalose metabolism (KEELING & al [17]). Instead, metabolites related to metabolic pathways of energy metabolism, including citric acid cycle (TCA), pentose phosphate pathway and mitochondrial electron transport chain were found here. A further survey on the key catalyzing enzyme genes involved in pathways of TCA and pentose phosphate pathway in *N. antheraeae* genome data. Six pentose phosphate pathway-related genes, including two 6-phosphogluconate dehydrogenase genes, one 6-phosphogluconolactonase gene, one glucose-6-phosphate 1-dehydrogenase gene, one epimeraseepimerase gene and one isomerase encoding genes, which played key roles in pentose phosphate pathway, were obtained. This indicated that pentose phosphate pathway was relatively complete in *N. antheraeae*, likely in *Nosema bombycis* (PAN & al [18]). Dramatically different parasitic conditions were speculated as the most possible causes for the absence of pentose phosphate metabolism in *Enterocytozoon bieneusi*.

Interestingly, no genes of TCA pathway was found in genome data of *N. antheraeae*. The acquisition of metabolites involved in TCA pathway might attribute to take in host-derived nutrients via membrande transporters. Futherly, many key enzymes genes related to glycolysis pathway, such as enolase, glucose-6-phosphate isomerase, 6-phosphofructokinase, fructose-bisphosphate aldolase, etc., were searched in *N. antheraeae* genome data. This indicated the exsitance of glycolysis, but the absence of TCA pathway implied that the amount of produced ATP might well below that of glucose completely oxidized. It would be interesting to uncover how about the spores to overcome this problem to obtain more ATP for meeting the demand of growth and reproduction. Fortunately, one ATP/ADP carrier protein was found in *N. antheraeae*, which was reported can obtain ATP or other energy molecules from host cells in obligate intracellular parasitic bacterium, including Rickettsia and Chlamydia (KRAUSE & al [19], TRENTMANN & al [20]). Thus, it was speculated that ATP/ADP carrier protein may involved in energy transportation to meet the need of *N. antheraeae* for energy.

Microsporidian mainly relied on trehalose metabolism to degradate trehalose to glucose, this can cause spore gemination through enhancement of inside osmotic pressure (UNDEEN & VANDERMEER [15]). It can also play an important role in spore stress-resistance to low temperature or dry conditions. In addition, the generated glucose by trehalose metabolism became one of the basic ways to provide energy for microsporidian metabolic activity (VIVARES & al [16]). At present, the main enzymes encoding genes involved in trehalose metabolism were identifed in many microsporidian including *Nosema bombycis*, *Nosema locustae*, *Nosema ceranae*, *Encephalitozoon cuniculi*, etc., except *Enterocytozoon bieneusi*. All of trehalose-6-phosphate synthases (TPS), trehalose-6-phosphate phosphatases (TPP) and UDP-glucose pyrophosphorylases (UGPA) possessed conserved domains and alike gene locus. Interestingly, microporidian parasitized in terrestrial or aquatic organisms showed distrinct modes of trehalose metabolism (DOLGIKH & SEMENOV [21]). In this study, TPS, TPP and trehalase encoding genes were obtained in *N. antheraeae* genome database. Meanwhile, trehalose was found in GC-MS-based metabonomics data. This indicated that *N. antheraeae*, which was parasitic in Tussah, may exhibite the similar mode of trehalose metabolism to *Nosema bombycis* and showed no obvious changes concentration of reducing sugar in spores during polar tube extruded (DOLGIKH & SEMENOV [21]).

Here, fourteen amino acids were identified and took almost 27% of the identified metabolites, including Glycine, Alanine, Lysine etc., they were employed as resources for protein sythnesis in the life cycle. However, genomic data showed that repertoire for the biosynthesis of amino acids in microsporidia was very restricted. For example, asparagine synthetase and serine hydroxymethyltransferase encoding genes were reported only in *Encephalitozoon cuniculi* genome (KEELING & al [17]). While, asparagine synthetase were identified here in *N. antheraeae* and serine hydroxymethyltransferase gene was also missing. The free amino detected in spores may be imported by parasite during their intracellular development. A further study to compare corresponding concentrations of major and minor metabolites during different developmental stages in microsporidia, can offer more details for confirming he presence of pathways in parasite metabolic system.

Conclusion

This work firstly analyzed important metabolic pathways in microsporidia, *Nosema antheraeae*, using GC-MS. The main metabolic pathways, which the detected metabolites participated in, included protein biosynthesis, ammonia recycling, urea cycle, galactose metabolism, alanine metabolism, arginine and proline metabolism, malate-aspartate shuttle, and so on. The results provided a new tool for understanding the metabolic pathways in spores. It may offer a new horizon for seaking pathogenic molecules during infection of spores on metabolomic levels. Next work would continually work on comparing concentrations of metabolites in spores under different developmental stages.

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References

1. J.S. XU, L.J. WANG, F.H. TANG, W. HUANG, Z.Y. ZHOU. The nuclear apparatus and chromosomal DNA of the microsporidian Nosema antheraeae. *J. Eukaryot. Microbiol*., 58(2): 178-180 (2011) .
2. Y. WANG, W. LIU, Y.R. JIANG, L. HUANG, M. IRFAN, S.L. SHI, R.S. YANG, L. QIN. Morphological and molecular characterization of Nosema pernyi, a microsporidian parasite in Antheraea pernyi. *Parasitol. Res*., 114(9):1-10 (2015) .
3. L.L.WANG, K.P. CHEN, Z. ZHANG, Q. YAO, G.T. GAO, Y. ZHAO. Phylogenetic analysis of nosema antheraeae, (microsporidia) isolated from chinese oak silkworm, Antheraea pernyi. *J. Eukaryot. Microbiol*., 53(4):310-313 (2006).

4. X.C. TANG, Z.R. HUANG, B. NING, C.Y. ZHANG. Detection of the spores of Nosema antheraeae by monoclonal antibody technique. *Acta. Sericologica. Sinica*., 25(4):221-224 (1999) .
5. J.J. GONG, J.S. XU, Z. LI, X.Q. DANG, L.L. WANG. Identification and Functional Analysis of a Long Pole Tussah of the Total Protein of Nosema antheraeae. *J. Chongqing Normal Univ.*, 31(3):26-30 (2014) .
6. A.A. VANYUSHKINA, G.Y. FISUNOV, A.Y. GORBACHEV, D.E. KAMASHEV, V.M. GOVORUN. Metabolomic analysis of three Mollicute species. PLoS One, 9(3): e89312 (2014) .
7. Q. HUANG, Y.X. TAN, P.Y. YIN, G.Z. YE, P. GAO, X. LU, H.Y. WANG, G.W. XU, Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. *Cancer Res*., 73(16): 4992-5002 (2013) .
8. G.A. BARDING-JR, S. BENI, T. FUKAO, J. BAILEY-SERRES, C. K. LARIVE. Comparison of GC-MS and NMR for metabolite profiling of rice subjected to submergence stress. *J. Proteome Res*., 12(2): 898-909 (2013).
9. Q.M.CHEN, X.Y. LIU, P. ZHAO, Y.H. SUN, X.J. ZHAO, Y. XIONG, G.W. XU, Q.Y. XIA. GC/MS-based metabolomic studies reveal key roles of glycine in regulating silk synthesis in silkworm, Bombyx mori. *Insect Biochem. Mol. Biol*., 57:41-50 (2015).
10. L.C. SALANȚĂ, S.A. SOCACI, M. TOFANĂ, E. MUDURA, C.R. POP, M. NAGY, A. ODAGIU. Characterization of volatile components in hop pellets using in-tube extraction GC-MS analysis. *Rom Biotechnol Lett*. 23(2): 13541-13550(2018).
11. D.L. YANG, G.Q. PAN, X.Q. DANG, Y.W. SHI, C.F. LI, P. PENG, B. LUO, M.F. BIAN, Y. SONG, C. MA, J. CHEN, Z.G. MA, L.N. GENG, Z. LI, R. TIAN, C.F. WEI, Z.Y. ZHOU, Interaction and assembly of two novel proteins in the spore wall of the microsporidian species Nosema bombycis and their roles in adherence to and infection of host cells. *Infect Immun*., 83(4): 1715-1731 (2015) .
12. Z.G. MA, C.F. LI, G.Q. PAN, Z.H. LI, B. HAN, J.S. XU, X.Q. LAN, J. CHEN, D.L. YANG, Q.M. CHEN, Q. SANG, X.C. JI, T. LI, M.X. LONG, Z.Y. ZHOU. Genome-wide transcriptional response of silkworm (Bombyx mori) to infection by the microsporidian Nosema bombycis. PLoS One, 8(12): e84137 (2013) .
13. Y. LI, X. WANG, Q.M. CHEN, Y. HOU, Q.Y. XIA, P. ZHAO. Metabolomics Analysis of the Larval Head of the Silkworm, Bombyx mori. *Int. J. Mol. Sci*., 17: 1460 (2016) .
14. K.K. PASIKANTI, P.C. HO, E.C. CHAN. Gas chromatography/mass spectrometry in metabolic profiling of biological fluids. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, 871(2): 202-211 (2008) .
15. A.H. UNDEEN, R.K. VANDER MEER. Microsporidian intrasporal sugars and their role in germination. *J. Invertebr. Pathol*., 73(3): 294-302 (1999) .
16. C.P. VIVARES, M. GOUY, F. THOMARAT, G. MERENIER. Functional and evolutionary analysis of a eukaryotic parasitic genome. *Curr. Opin. Microbiol*., 5(5): 499-505 (2002) .
17. P.J. KEELING, N. CORRADI, H.G. MORRISON, K.L. HAAG, D. EBERT, L.M. WEISS, D.E. AKIYOSHI, S. TZIPORI. The reduced genome of the parasitic microsporidian Enterocytozoon bieneusi lacks genes for core carbon metabolism. *Genome Biol. Evol*., 2(1): 304-309 (2010) .
18. G.Q. PAN, J.S. XU, T. LI, Q.Y. XIA, S.L. LIU, G.J. ZHANG, S.G. LI, C.F. LI, H.D. LIU, L. YANG, T. LIU, X. ZHANG, Z.L. WU, W. FAN, X.Q. DANG, H. XIANG, M.L. TAO, Y.H. LI, J.H. HU, Z. LI, L.P. LIN, J. LUO, L.N. GENG, L.L. WANG, M.X. LONG, Y.J. WAN, N.J. HE, C. LU, P.J. KEELING, J. WANG, Z.H. XIANG, Z.Y. ZHOU. Comparative genomics of parasitic silkworm microsporidia reveal an association between genome expansion and host adaptation. BMC Genom., 14(1): 186 (2013) .
19. D.C. KRAUSE, H.H. WINKLER, D.O. WOOD, Cloning and expression of the Rickettsia prowazekii ADP/ATP translocator in Escherichia coli. *P. Natl. Acad. Sci*., 82(9): 3015-3019 (1985) .
20. O. TRENTMANN, M. HORN, A.C.T. VAN SCHELTINGA, H.E. NEUHAUS, I. HAFERKAMP. Enlightening energy parasitism by analysis of an ATP/ADP transporter from chlamydiae. PLoS Biol., 5(9): e231 (2007) .
21. V.V. DOLGIKH, P.B. SEMENOV. Trehalose catabolism in microsporidia Nosema grylli spores. *Parazitologiia*, 37(4):333-342 (2002).