# Rapid Screening of Expressed Genes of *Trametes gallica* by cDNA Microarray*

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**Abstract** Effort was made to screen expressed genes of a white-rot fungus *Trametes gallica* by using cDNA microarray from *Phanerochaete chrysosporium*, a well known model strain used for studying lignin-degrading systems. The results showed that there were only 172 positive clones detected on the microarray and 95.9% of the clones had a ratio of Cy-5/Cy-3 from 0.5 to 2.0. However, only 3 and 4 clones with the Cy-5/Cy-3 ratio over 2.0 and lower than 0.5 respectively, were found, which are corresponding to 5- and 12-day cultures of *T. gallica*. One hundred and twenty-two clones were randomly selected and sequenced, of which 118 clones could be perfectly located in the genome of *P. chrysosporium*. The result indicates that there is obvious sequence difference between probes from *T. gallica* and inserts on the chip from *P. chrysosporium*, exhibiting far relative between these two fungi. Two interesting clones have been obtained from homology comparison, one corresponding to a fragment of peroxidase lpoB gene of *P. chrysosporium* and the other encoding a sort of heat shock protein.

**Key words** cDNA microarray, *Trametes gallica*, *Phanerochaete chrysosporium*, homology analysis

Developed early in 1990s, the novel gene chip technology has been being widely exploited for analysis of expression patterns from diverse tissues[1,2]. The present cDNA microarray technology has made it possible for efficient and high throughput analysis of differential gene expressions in cell lines or tissues. In recent years, a lot of temporal-specifically expressed genes were cloned by suppression subtractive hybridization (SSH) and cDNA microarray[3~7]. The Genome-sequencing Project of *Phanerochaete chrysosporium* (a well known white-rot fungus model strain used for studying lignin-biodegrading system) has resulted in vast sequence information, which will enable the efficient comparison of sequence data from other microorganisms. White-rot fungus *Trametes gallica*, with strong capacity to deplete lignocellulose, is one of the main bio-degraders of poplars[8]. It is significant to study the genetic constituent and the secondary metabolic mechanism of the strain. Our recent results show that nitrogen starvation impels the secondary metabolism accompanied by peroxidase production in *T. gallica*, especially when the strain is grown in shallow stationary culture. In this paper we investigated the differential gene expression of *T. gallica* grown in the N-limited medium for 5 and 12 days to reveal the feasibility to screen the expressed genes in the fungus by using cDNA microarray made from another white-rot fungus *P. chrysosporium*. The results showed that there are some genes with homologous sequences between these two fungi.

## 1 Materials and methods

### 1.1 Organism and cultivation conditions

*Trametes gallica*, which was previously isolated from decayed wood of a *Populus* sp. and collected at Biology Department of Heze Teacher's College, Heze, China[8], was used in this study and maintained at 4℃ on potato dextrose agar (PDA) slants. Subculture was routinely made every 2 months. Three mycelia disks were punched from *T. gallica* grown on PDA plate for 6 days at 26℃ and used as inoculum for each culture (PDA on the disks was removed as much as possible). All shallow stationary cultures were grown in air. The N-limited medium (2.2 mmol/L, C/N = 303) for cultivation of *T. gallica* was based on the one described by Tien and Kirk[9], with exception that glucose content was modified and that $MnSO_4$ was reduced to half of the final concentration. After 5 and 12 days of incubation at 26℃, the mycelia were harvested for the extraction of total RNA, respectively.

### 1.2 Preparation of total RNA

Two total-RNA isolates of *T. gallica* cultured for 5 and 12 days on the N-limited medium were extracted respectively[10]. The RNA quality was assessed by formaldehyde agarose gel electrophoresis and quantitated spectrophotometrically.

### 1.3 Preparation of fluorescent dye-labeled DNA and hybridization

RNA was further purified with an RNeasy midi kit (Qiagen) according to the manufacturer's instructions. The first- and second-strand cDNA synthesis was performed by using the cDNA synthesis system kit (TaKaRa, Dalian, China). Fluorescent dye (Cy-5- and Cy-3-dCTP) labeled DNA was produced through single primer amplification (SPA) reaction[11]. Labeled DNA solution was incubated at 95℃ for 3 min and cooled to room temperature before being hybridized to

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the cDNA microarray, which contained 2 596 cDNA fragments from a SSH (suppression subtractive hybridization) library (made in the laboratory of author) of *P. chrysosporium* BKMF-1767 grown on shallow N-limited culture for 2 and 3 days. Hybridization was performed at 42℃ overnight. After hybridization, slides were washed in 0.1% SDS, 0.6 × SSC at 50℃ for 5 min and 0.03% SSC for 5 min at room temperature. Then the slides were centrifuged to dryness in 50 ml conical tubes at 1 000 r/min for 1 min. A fluorescence-exchanging labeling experiment was also performed for the two RNA samples.

### 1.4 Imaging and data analysis

The hybridized array was scanned by using a laser confocal scanner ScanArray Express (Parckard Bioscience, Kanata, OT, USA). Internal controls on the array were chosen to balance the scanner setting. Images obtained were analyzed by a computer program GenePix Pro 3.0 (Axon Instruments, Foster City, CA, USA). A space and intensity-dependent normalization based on a Lowess program was employed here. Spots whose ratio lay outside the 95% confidence interval were determined to be differentially expressed genes (Capital Biochip Corporation, China).

### 1.5 DNA sequencing and homology analysis

Dideoxynucleotide chain termination method was used for DNA sequencing (United Gene Institute, Shanghai, China) and the BLAST program was employed for the sequence homology analysis.

## 2 Results and discussion

### 2.1 Extraction of total RNA

The clear 26 S, 18 S and 5.8 S bands were observed after the denatured formaldehyde gel electrophoresis, and the brightness of 26 S band was obviously stronger than that of 18 S band (Figure 1).

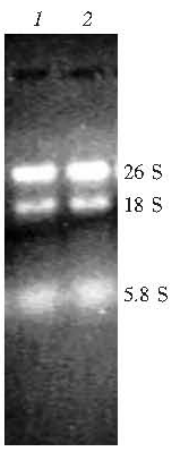


**Fig. 1 Denatured gel electrophoresis of the total RNA extracted from *T. gallica* grown for 5 (lane *1*) and 12 days (lane *2*).**

Spectrophotometrically qualified ratios ($A_{260}/A_{280}$) of 5- and 12-day RNA were 1.875 and 1.869, respectively, suggesting that the total RNA is appropriate for the subsequent hybridization.

### 2.2 Analysis of the hybridized microarray

A set of 2 596 cDNA clones was obtained from SSH of *P. chrysosporium* grown on N-limited medium for 2 and 3 days. The cDNA insert of each clone was amplified with PCR and spotted on a microarray using robotic printing. Multiple yeast housekeeping genes and randomly selected cDNAs were also printed on the same array to serve as internal controls. The microarray was subsequently hybridized with *T. gallica* cDNA probes labeled with fluorochromes. Probes were prepared from two total RNA isolates of *T. gallica* cultivated for 5 and 12 days, respectively. An example of such hybridization is shown in Figure 2. The 5-day cDNA probe was labeled with Cy-3 fluorochrome (green) while the 12-day cDNA probe with Cy-5 fluorochrome (red). Green and red colors indicate high-level gene expression of *T. gallica* at 5-day and 12-day, respectively, while the yellow color indicates equal expression. A fluorochrome-counterchanged hybridization was also performed in which the 5-day cDNA probe was labeled with Cy-5 fluorochrome (red) and the 12-day cDNA probe with Cy-3 fluorochrome (green).

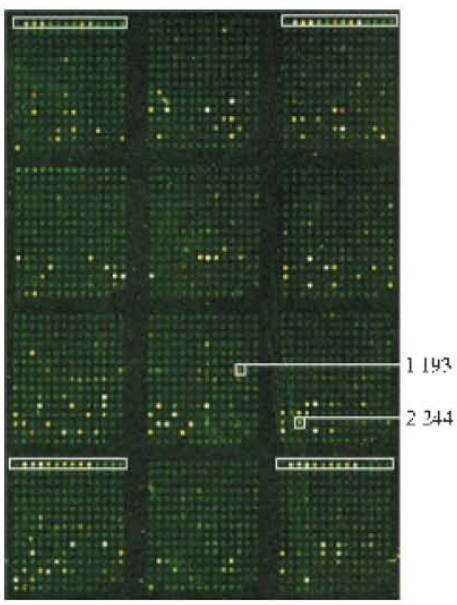


**Fig. 2 Microarray hybridized with fluorescence labeled cDNA probes**

A cDNA microarray was hybridized with fluorescence labeled cDNA probes prepared from 5 -(green) and 12-day (red) mRNA of *T. gallica*. Red and green colors indicate relative over-expression in *T. gallica* at 12-day and 5-day respectively. Yellow color indicates equal expression at both times. Some internal controls are outlined with boxes. The clones 1 193 and 2 244 are also indicated. The microarray picture from the fluorochrome-counterchanged hybridization is not shown here.

Comparison of hybridization data shows that there are only 172 positive clones detected on the chip. Only 3 (Cy-5/Cy-3 < 0.5) and 4 (Cy-5/Cy-3 > 2.0) temporal-specific expression clones, which are corresponding to 5- and 12-day cultures of *T. gallica* respectively, were found, and none of these 7 clones show the same ratio level in the microarray hybridization of *P. chrysosporium* (the same array hybridized with cDNA probes prepared from the 2- and 3-day mRNA of *P. chrysosporium*). Accounting for 95.9% of the total detected clones, 165 clones are within the ratio (Cy-5/Cy-3) from 0.5 to 2.0, in which 96 clones (58.2%) have the same ratio range as that from the hybridization of *P. chrysosporium*. The result suggests that these clones are likely from the housekeeping genes of the two strains simultaneously, and also indicates that the genetic constituent and function similarity exist in both strains to a certain extent.

The hybridizations were scanned and quantitative information was obtained. The scatter-plot, with its coordinate axes $X$ and $Y$ representing the relative intensities of Cy-3 and Cy-5 labeled samples respectively, was from the software analysis of the hybridized microarray (Figure 3). Spots scattered on the 45° line, which are with the ratio (Cy-5/Cy-3) of 1, represent equally expressed genes. The farther the spots from the 45° line, the more differentially the corresponding genes express. Spots scattered between the $Y$-coordinate and the upper broken line, with ratios of larger than 2.0, represent up-regulated genes, while those down-regulated genes with ratios of less than 0.5 are located between the $X$-coordinate and the lower broken line.

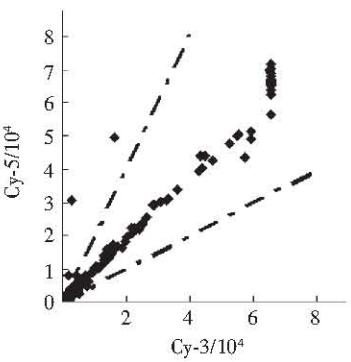


**Fig. 3 Scatter-plot of the hybridization signals**

Clones 2 238, 1 863, 2 460 and 1 193 located between the coordinate axis $Y$ (Cy-5) and the upper broken line, were differentially expressed genes in *T. gallica* when grown in N-limited medium for 12 days.

## 2.3 Analysis of sequence homology

Among 172 positive clones, 122 clones were randomly selected and sequenced, and 118 clones (accounting for 6.7% of the 2 596 cDNA fragments on the chip) could be perfectly located in the genome of *P. chrysosporium*, indicating that obvious sequence homology exists between some probes made from *T. gallica* and some inserts on the chip from *P. chrysosporium*. The reason why approximately 93.4% of the 2 596 cDNA fragments have no hybridization signals may lies in the fact that the two fungi have low sequence homology in their genomic constituents, and that some genes of *T. gallica*, such as regulator genes, transcribe at very low expression level. The 118 sequenced fragments were classified into three groups, i. e., the unique, the completely paralleled and the partially paralleled sequences. Forty-seven clones with different sequences were eventually obtained, of which 23 clones were found with unknown functions or no high homology when compared to known sequences in the GenBank database, probably representing novel genes. The results are summarized in Table 1.

The clones, which are identical to and partially paralleled to clone 2 559 in sequence composition, proportion 39.3% in the 122 sequenced clones. The clones from partial sequence of 5.8 S rRNA gene (e. g., clone 2 118) also accounts for 8.2%, suggesting that they were possibly resulted from the remains of rRNAs during the mRNA purification and the SSH library construction of *P. chrysosporium* (i. g., perhaps, the regions rich in base A in rRNAs led to the relating reverse transcription). The result simultaneously demonstrates that the rRNA genes of the two fungi are of a high homology. It has also been found that the different parts of the same sequence for a few clones can be located separately in different Scaffolds of *P. chrysosporium*, which may be resulted from the recombination of different *Rsa* I cDNA fragments of *P. chrysosporium* during SSH process (i. e., from the random ligation of *Rsa* I cDNA fragments).

The housekeeping genes encode some important protein factors or enzymes that involve in transcription, translation and saccharide metabolism. The result shows that most clones with the ratios (Cy-5/Cy-3) from 0.5 to 2.0 are housekeeping genes, for example, clones 1 318 and 1 925 encode ribosomal proteins which involve in composing of ribosomes, clones 1 899 and 757 encode translation initiation and elongation factors, respectively. All these clones are related to translation process (Table 1). Some 5- and 12-day specifically expressed genes (with Cy-5/Cy-3 < 0.5 and > 2, respectively) are also listed in Table 1. According to the Blast analysis, an interesting clone 2 244 has been obtained as a fragment of peroxidase lpoB and lignin peroxidase lpoA genes of *P. chrysosporium*, indicating that these genes exist in

the two strains and have started expression in *T. gallica* grown on N-limited medium for only 5 days. Another interesting clone is the clone 1 193, with insert size of 420 bp, showing a ratio (Cy-5/Cy-3) of 2.1. It corresponds to the gene encoding a sort of heat shock protein of *Coriolus versicolor* (*Trametes versicolor*) and expresses differentially when N source is depleted out. This property can be used to study the secondary metabolic mechanism of *T. gallica*, especially for the lignin-degrading enzyme systems under environmental stress. Heat shock proteins, which involve in a variety of cellular activities, have been widely studied so far[12~14]. Of course, it is necessary to identify the exact functions of the above cloned fragments. Furthermore, according to the hybridization result, it is possible to screen some genes of interest from cDNA library of *T. gallica* by using cDNA probes made from *P. chrysosporium*. All these works are still in progress. In the present study, the reason why none of the known regulator genes in the GenBank was found may be due to the far relative of the two strains and the relatively low copy number of the corresponding mRNAs. Optimization of hybridization condition to increase the ratio of signal to noise will improve the ability to identify the clones corresponding to mRNAs with low copy number. In conclusion, the microarray technology can be applied to rapid screening and identifying many genes for *T. gallica* although the cDNA chips are made from another lignin-degrading white-rot fungus such as *P. chrysosporium*.

**Table 1 The expressed clones with known functions in GenBank database**

| Clones | Cy-5/Cy-3 | Insert size/bp | Homology |
|---|---|---|---|
| 1 913 | 0.44 | 211 | JUN-activation-domain-binding protein homolog [*Oryza sativa*]. T02934 |
| 624 | 0.45 | 829 | ATP-binding cassette. NP_ 036965.1 |
| 2 244 | 0.48 | 75 | Peroxidase lpoB and lignin peroxidase lpoA genes [*P. chrysosporium*]. M37701.1 |
| 2 441 | 0.66 | 180 | Fructose-bisphosphate aldolase, FBAI [*Kluyveromyces lactis*]. CAC29023.2 |
| 2 291 | 0.73 | 426 | Ubiquitin conjugating enzyme UBC1 [*Glomerella cingulata*]. AAC39499.1 |
| 1 318 | 0.87 | 532 | 60 S ribosomal protein L4 [*Chlamydomonas* sp. HS-5]. BAA78600.1 |
| 1 925 | 0.91 | 341 | Ribosomal protein, small subunit RPS-5 [*Caenorhabditis elegans*]. NP_ 502077.1 |
| 1 980 | 0.93 | 289 | CoPCNA [*Coprinopsis cinerea*]. BAB83687.1 |
| 1 899 | 0.96 | 334 | Eukaryotic translation initiation factor 4C. NP_ 001403.1 |
| 1 739 | 0.98 | 282 | Tyrosine-protein kinase transforming protein FGR. P00544 |
| 1 936 | 1.00 | 261 | Chitin deacetylase [*Schizophyllum commune*]. AF271216_ 1 |
| 1 408 | 1.01 | 84 | Phospho-2-dehydro-3-deoxyheptonate aldolase. NP_ 010320.1 |
| 2 045 | 1.02 | 265 | GTP-binding nuclear protein spi1 [*Schizosaccharomyces pombe*]. NP_ 596827.1 |
| 718 | 1.05 | 115 | Putative ubiquitin ligase [*Schizosaccharomyces pombe*]. NP_ 594902.1 |
| 2 118 | 1.06 | 218 | 5.8 S rRNA gene [*Tricholoma matsutake*]. U62964.1 |
| 2 467 | 1.06 | 171 | Symbiosis-related protein [*Laccaria bicolor*]. AAB53650.1 |
| 2 196 | 1.11 | 195 | Putative histone [*Agaricus bisporus*]. CAC03460.1 |
| 2 412 | 1.13 | 315 | Polyubiquitin [*Botryotinia fuckeliana*]. AAC15225.1 |
| 1 411 | 1.14 | 75 | Peroxiredoxin 4 [*Dictyostelium discoideum*]. AAM44383.1 |
| 757 | 1.16 | 222 | Translation elongation factor 1a [*Schizophyllum commune*]. CAA64399.1 |
| 2 559 | 1.68 | 156 | 18 S ribosomal RNA gene [*P. chrysosporium*]. AF026593.1 |
| 2 248 | 1.90 | 441 | Heat stress protein 80-1[*Neurospora crassa*]. AAB26285.1 |
| 1 193 | 2.10 | 420 | Heat shock protein [*Coriolus versicolor*]. BAA76591.1 |
| 2 238 | 10.50 | 146 | 88 ku immunoreactive mannoprotein [*Filobasidiella neoformans*]. AF480842_ 1 |

The Identities (Blastn) for clones 2 559 and 2 118 are 100% and 96%, respectively. All the Identities used for BlastX are more than 81% except that for clones 624 and 1 318 with the Identities 68% and 67%, separately. The clones, which possibly represent novel genes, were not shown here.

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# 用 cDNA 微阵列技术快速筛选粗毛栓菌的表达基因*


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摘要 利用 cDNA 微阵列技术快速筛选具有较强降解木质纤维素能力的白腐真菌粗毛栓菌（*Trametes gallica*）的表达基因. 利用木质素生物降解模式菌株黄孢原毛平革菌（*Phanerochaete chrysosporium*）的 cDNA 制备研究所用微阵列. 在含有2 596 个 cDNA 片段的芯片上共检测到 172 个阳性克隆，其中有 165 个克隆的荧光信号比值（Cy-5/Cy-3）在 0.5 和 2.0 之间，占所检测阳性克隆数的95.9%. 对应于在限氮条件下生长 5 天和 12 天的粗毛栓菌培养物，分别有 3 个和 4 个时序特异性差异表达基因. 随机挑取 122 个克隆进行测序和序列比对，发现所测序列中有 118 个能够很好地定位于黄孢原毛平革菌的基因组上. 结果显示，粗毛栓菌与黄孢原毛平革菌在表达序列上存在较大差异，表明这两种真菌之间存在着较远的亲缘关系. 通过同源性比对分析，发现 2 个令人感兴趣的克隆，一个对应于黄孢原毛平革菌过氧化物酶基因 lpoB 的部分片段，另一个为编码一种热激蛋白的基因.



关键词 cDNA 微阵列，粗毛栓菌，黄孢原毛平革菌，同源性分析

学科分类号 Q786


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