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Microarray analysis of gene expression profile induced by the biocontrol yeast *Cryptococcus laurentii* in cherry tomato fruit

Feng jiang [a,b], Xiaodong Zheng [a], Jishuang Chen [b,*]

[a] Department of Food Science and Nutrition, Zhejiang University, Hangzhou, 310029, People's Republic of China
[b] Institute of Bioengineering, Zhejiang Sci-Tech University, Hangzhou, 310018, People's Republic of China

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ABSTRACT

To obtain an overall view on transcript modification during the cherry tomato fruit responding to biocontrol yeast *Cryptococcus laurentii*, we performed a microarrary analysis, using Affymetrix Tomato Genechip arrays, representing approximately 10,000 genes. The results showed that 194 and 312 genes were up- or down-regulated, respectively, more then ten time fold (log2 change ratio≥2 or log2 change ratio≤−2) in biocontrol yeast treated tomato fruit as compared with control fruits. Those up-regulated included genes involved in metabolism, signal transduction, and stress response. Conversely, genes related to energy metabolism and photosynthesis were generally down-regulated. Our results suggest that biocontrol yeast treatment induces fruit resistance response, suppresses energy metabolism and photosynthesis. A number of transcripts encoding proteins/enzymes which are known to be up-regulated under some biotic and abiotic stress are also up-regulated after the applying biological control yeast to cherry tomato fruit. The expression of these proteins might increase the fruit resistance towards postharvest pathogen infection and damage.

1. Introduction

Biological control postharvest diseases using microbial antagonists has emerged recently as a promising alternative to the use of synthetic fungicides (Wilson and Wisnieswski, 1989; Wisniewski et al., 1991), either along or as part of integrated pathogens management to reduce fungicides use. Several biocontrol agents, which include yeast and bacteria have been investigated and shown to protect against a number of postharvest pathogens on a various harvested commodities. Up to now, four antagonistic microorganisms—two yeasts (*Candida oleophila*, *Cryptococcus albidus*) and two strains of the bacterium *Pseudomonas syringae*—are commercially available (Janisiewicz and Korsten, 2002).

Antagonistic yeasts have been selected mainly for their activity that does not generally depend on the production of toxic metabolites, which could have a negative environmental or animal toxicological impact, for their ability to colonize and grow rapidly in surface wounds, and because they are generally poorly sensitive to fungicides. Mechanisms which have been reported to play a role in the biocontrol activity of antagonistic yeasts include competition for space and nutrients, activation of host defenses, and production of extracellular depolymerases which putatively act on pathogen cell walls. Competition for space and nutrients has been demonstrated as a major mechanism in the antagonism of biocontrol yeasts against postharvest fungal pathogens (Castoria et al., 1997, 2001; Droby and Chalutz, 1994; Droby et al., 1989; Jijakli and Lepoivre, 1998; Wilson and Wisniewski, 1994).

There is growing evidence to support that antagonistic yeasts are capable of inducing resistance mechanisms in the host tissue. Ippolito et al. (2000) show that the yeast *Aureobasidium pullulans* has ability to control postharvest decay of apple caused by *B. cinerea* and *P. expansum* and has capable of inducing the accumulation of chitinase, β-1,3-glucanase, and peroxidase in apple fruit. Tian et al. (2007) showed that after jujube fruit treated by *C. laurentii* the expression of *Glu-1* from noticeably enhanced with increased concentrations of *C. laurentii*, suggesting that β-1,3-glucanases may play a role in defense responses to fungal pathogens. Arras (1996) report that antagonistic yeasts can induce the accumulation of the phytoalexin, scoparone, in citrus peel. And also antagonistic yeasts can induce the deposition of structural barriers in apple fruit (El-Ghaouth et al., 1998).

Recently, we have isolated a yeast, *Cryptococcus laurentii*, from the surface of tomato fruit and we showed that it was effective involved in controlling decay of apple, orange, peach and sweetcherry (Zhang et

Abbreviations: ACC, 1-Aminocyclopropane-1-carboxylic acid; ACS, 1-Aminocyclopropane-1-carboxylic acid synthase; DEPC, Diethypyrocarbonate; ETR, Ethylene receptor; EXP, Expansin; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; MAPK, Mitogen-activated protein kinases; NYDA, Nutrient yeast dextrose agar; NYDB, Nutrient yeast dextrose broth; PG, Golygalacturonase; PR, Pathogenesis-related; ROS, Reactive oxygen species; SAR, Systemic acquired resistance; XET, Xyloglucan endotransglycosylases.

* Corresponding author. Tel.: +86 57186971167; fax: +86 57186971139.
E-mail address: chenjs@zstu.edu.cn (J. Chen).

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al., 2003, 2005a,b; Yu et al., 2008). *In vivo* studies revealed that *C. laurentii* was capable of inducing accumulation of resistance related enzymes (Tian et al., 2007). In order to maximize the potential use of *C. laurentii* for the control of postharvest decay of fruit, an understanding of its mode of action is necessary. Little has been published regarding its ability to induce host defense responses. In this study, we used Affymetrix Tomato Genechip microarrays to evaluate changes in gene expression in response to *C. laurentii* in cherry tomato fruit. Our goal was to identify a suite of genes that are regulated by biocontrol yeast and use the results obtained to better understand the molecular mechanisms underlying tomato fruit postharvest induced resistance. Approximately 50% of the genes represented on the array were regulated by *C. laurentii* in tomato fruit. Results here demonstrate that plants may use some common molecular mechanisms (e.g. signal transductions) in response to various stress conditions.

2. Materials and methods

2.1. Plant material and yeast inoculation

Cherry tomato fruits were collected at MG4 (mature green) stage from tomato plants grown under greenhouse conditions. Yeasts were grown on nutrient yeast dextrose agar (NYDA) medium (containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar in 1 l distilled water) at 28 °C. Yeast cells were grown in 250 ml liquid cultures containing 50 ml of nutrient yeast dextrose broth (NYDB) for 16 h at 28 °C on a rotary shaker at 200 rpm. Cells were then collected by centrifuging at 7000 g for 10 min and washed twice with sterile distilled water to remove the growth medium. Cell numbers were determined with hemocytometry and diluted with sterile distilled water as required.

Fresh fruits were surface sterilized by incubation for 10 min in a 1‰ NaClO solution and subsequent washing in distilled water three times. Fruits were air dried and wounded by making puncture with a yellow tip at one site around the equator. 5 µl of suspensions of 1×10^8 yeast/ml were inoculated in the wound. Control fruits were either intact (ripening control) or inoculated with 5 µl of sterilized water (wounding control). Inoculated fruits and the corresponding ripening and wounding controls were maintained at 20 °C and 85–90% relative humidity. Tissue from pericarp around the inoculation site was collected 24 h after inoculation, frozen in liquid nitrogen immediately, and stored at −80 °C. Pericarp from control fruits were also collected and processed in the same way.

2.2. RNA extraction and microarray analysis

Total ribonucleic acid (RNA) was isolated from both tomato fruit pericarp of *Cryptococcus laurentii* treated as well as control tomato fruit using TriZol reagent (Invitrogen). Frozen fruit pericarp (approximately 1 g) were homogenized with a mortar and pestle in liquid nitrogen. The ground powder was transferred to a polypropylene tube, and 5 ml of TriZol reagent was added. The homogenates were shaken with vortex for 30 s, and plant debris was removed after centrifugation (12,000 ×g) for 10 min at 4 °C. Chloroform–isoamylalcohol (24:1) (2 ml) was added to the supernatant and was centrifuged (12,000 ×g) for 10 min at 4 °C after mixing. Total RNAs were precipitated from the aqueous phase with 0.6×volume of isopropanol by centrifugation (12,000 ×g) for 10 min at 4 °C, and the precipitated RNAs were dissolved in DEPC-treated water. The RNA samples were further purified using RNeasy mini plant kit (Qiagen) for microarray analysis. The RNA yield and quality were determined by spectrophotometry at 260 and 280 nm. The RNA integrity was checked on 1.5% (w/v) agarose/formaldehyde gel. For microarray analysis, total RNAs were processed for use on Affymetrix Tomato Genechip arrays according to the manufacturer's protocol. In brief, 15 µg of total RNAs were used in a reverse transcription reaction to generate first-strand cDNAs, using the SuperScript III (Invitrogen) with random primers. After second-strand synthesis, biotin-labeled target complementary RNAs (cRNAs) were prepared using the BioArray high-yield RNA transcript labeling kit in the presence of biotinylated UTP and CTP. After purification and fragmentation, 15 µg of cRNAs were used in a 300 µl hybridization mixture containing added hybridization controls. A total of 200 µl of the mixture was hybridized on arrays for 16 h at 45 °C. Standard posthybridation wash and double-stain protocol were applied on an Affymetrix GeneChip fluidics station 450. Arrays were scanned on an Affymetrix GeneChip scanner 2500.

2.3. RT-PCR analysis

Total RNAs isolated as described above were used for RT-PCR verification, with fruit tissues treated with biocontrol yeast *Cryptococcus laurentii* 0 h, 24 h to 48 h, respectively. After treatment with RNase-free DNase followed by phenol extraction and ethanol precipitation, total RNAs were reverse transcribed in 20 µl reaction using SuperScriptIII (GIBACO) with the oligo$(dT)_{15}$ at 50 °C 60 min. For PCR amplification, 2 µl RT mix was used as template. PCR reaction was carried out in 25 µl with 0.5 U of rTaq DNA polymerase (TaKaRa). The PCR program was as follows: 2 min at 94 °C 25 cycles of 30 s at 94 °C, 45 s at the optimal annealing temperature (50 to 65 °C), and 60 s at 72 °C, followed by 8 min at 72 °C. The amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal control. The PCR products were checked with 1.5% agarose gel in 1×TAE with EtBr. Sets of gene-specific primers used show in Table 1.

3. Results

3.1. Microarray analysis of gene expression in tomato fruit after inoculation with C. laurentii

To obtain an overall picture of gene regulation during biocontrol yeast treated, two independent microarray analyses were performed. To reduce experimental variation, two sets of twenty fruits were harvested from biocontrol yeast-treated and untreated (wounding control) fruit, respectively, at 24 h post treatment. Then total RNAs were prepared from the replicates of the pooled samples. RNAs were analyzed using the Affymetrix Tomato Genechip containing 10,038 tomato probe sets representing 9254 transcripts. The Affymetrix Microarray Suite Software (MAS version 5.0) was applied for analyzing the reliability of signals for each probe set (Wang et al., 2003). Changes in expression of all the probe sets on the microarray are depicted in Fig. 1. In this study, 4210 transcripts were reliably detected in microarray analyses. Changes in RNA levels in response to *C. laurentii* treatment (i.e. induced or repressed) were assessed using Affymetrix

Table 1
Primers used for RT-PCR

Signal ratio (Log2)	Gene name	Primer set	Expected size (bp)
3.1	*Le*MKK1	5′-GTGCCTATGATTACAAAAGCGACA-3′ 5′-TTGCCAATGGAGGTCCTGCGG-3′	333
5.7	β-1,3-glucanase	5′-GCTTTCTCGGACTACCTTCTTTA-3′ 5′-GGAATGATGGGGAACAACTT-3′	804
7.6	PR5-like protein	5′-GTGATTGTGGTGGAGTCTTGGA-3′ 5′-ATAGGCATCGGGACATCTTTG-3′	346
−7.9	*Le*XET2	5′-CGTAGTGGTGGCTTTTGGTGGTA-3′ 5′-CTCTCTATCTCCCTTGCCTTGTG-3′	337
Control	GAPDH	5′-CGGCTCCATTTTCCTTCT-3′ 5′-GGGATTTCCTCTGGGTTTC-3′	299

An oligonucleotide primer set was designed for selected genes using Primer Premier 5.0 and was used for RT-PCR analysis.
The expected length for the PCR product for each gene is indicated.