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Transcript profiling analysis of *Rhodosporidium paludigenum*-mediated signalling pathways and defense responses in mandarin orange

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ABSTRACT

To investigate the basis of inducible resistance response in postharvest mandarin orange, cDNA microarray and high-performance liquid chromatography were performed to study transcriptional and metabolic changes in *Rhodosporidium paludigenum* strain treated fruit. The microarray data mining revealed that *R. paludigenum* activated transcription of genes important for plant hormones, signalling transduction, stress and defensive responses in orange peel tissue. Moreover, up-regulation of phenylalanine and tyrosine metabolism, phenylpropanoids biosynthesis, and alkaloid biosynthesis I, were observed at the transcription level. Conversely, large amounts of genes involved in starch metabolism, oligosaccharide and glycoside metabolism were markedly repressed by *R. paludigenum* treatment. Activation of phenylpropanoids biosynthesis pathway was correlated with the increasing production of phenolic acids and their subsequent metabolite lignin, indicating antifungal metabolites indeed contributed to biocontrol yeast enhanced fruit protection. Our findings provide an important basis for understanding the mechanisms of resistance induction in mandarin orange, as well as for reducing postharvest losses.

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1. Introduction

Postharvest diseases are responsible for substantial losses in harvested fruits during transportation and storage because fruits are natural hosts for fungal pathogens. Biological control of postharvest decays using microbial antagonists has emerged as an alternative approach that reduces the use of fungicides, and avoids the potential harmful effects of these chemicals on the environment and human health (Janisiewicz & Korsten, 2002). Several antagonist-based biocontrol products are currently commercially available for postharvest use, such as Bio-Save™ 10 LP and 11 LP (based on *Pseudomonas syringae*, registered in USA), Shemer™ (based on *Metschnikowia fructicola*, registered in Israel), Candifruit™ (based on *Candida sake*, registered in Spain), Pantovital (based on *Pantoea agglomerans*, registered in Spain), and Boni-Protect® (based on *Aureobasidium pullulans*, marketed in Germany). Despite their rapid market growth, these products only constitute a small fraction of agricultural chemical sales for postharvest disease management (Fravel, 2005). Many constraints and obstacles, such as relative lower decay control efficacy and stability, narrow application spectrum and higher cost than fungicides, have to be overcome before these products can be used as a practical control strategy, and more research is needed in many aspects of postharvest biocontrol.

A deeper understanding of fruit responses to microbial antagonists within a wound or on the fruit surface is essential in understanding the biology of postharvest biocontrol (Droby, Wisniewski, Macarisin, & Wilson, 2009). Enhanced disease resistance in fruits can be achieved by many potential antagonistic microbes that resist pathogen invasion (Droby et al., 2002; Lu et al., 2013). Interestingly, these processes are hypothesised to share many features with the defense mechanisms that are induced in actively growing plant tissues (Castoria & Wright, 2010). In plants, elicitors are produced and released by microbes once contact has been established; the host responds with transient production of signalling molecules and succeeding defense responses, such as the accumulation of pathogenesis-related (*PR*) proteins, antimicrobial compound biosynthesis, and localised cell-wall reinforcement (Dangl & Jones, 2001). Nevertheless, little information has been gathered on these possible mechanisms involved in postharvest biology. These dynamic processes are believed to have essential functions in the resistance response of stored fruits with non-pathogenic yeasts or bacteria.

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Signalling messengers are small molecules whose concentrations change in response to internal cellular demands or external environmental stimuli, providing an intracellular link for the regulation of plant growth and survival (Bostock, 2005). Amongst these signalling messengers, plant hormones salicylic acid (SA), ethylene, jasmonic acid (JA), and its derivatives are recognised as some of the major signalling compounds that govern plant immunity (Pieterse, Van der Does, Zamioudis, Leon-Reyes, & Van Wees, 2012). The early activation of genes involved in phytohormone biosynthesis can lead to appropriate transcriptome changes (Garcia-Brugger et al., 2006). Each of these hormones activates a set of defense genes that encode antimicrobial proteins or components in response to predation and pathogen attack in fruits. For instance, the *PR* genes *PR-1*, *PR-2*, and *PR-5* require SA signalling for activation, whereas the plant defensin genes *PDF1.2*, *PR-3*, and *PR-4* are activated via the JA-dependent pathway (Thomma et al., 1998). However, the function of these genes and compounds in biocontrol agent-induced disease resistance in fruits has yet to be established, even if the application of SA and JA appears to improve fruit protection (Castoria and Wright, 2010). Furthermore, other plant hormones, such as gibberelic acid (GA) and indole-3-acetic acid (IAA) could also induce defense responses and provide host resistance to necrotrophs.

Secondary metabolites play an important function in plant defense mechanisms via direct toxic effects and the active and rapid deposition of barriers, such as lignin (Bennett & Wallsgrove, 1994). The production of secondary metabolites correlates with the earliest onset of grapefruit disease resistance in response to wounding or antagonist *Candida famata* (Arras, 1996). Phenolic acids, one main group of secondary metabolites enriched in orange fruit peel, are derived from cinnamic acid and phenylalanine via the action of the branch point enzyme phenylalanine ammonia-lyase (PAL) between primary and secondary metabolism. Ethylene mediated stimulation of *PAL* and its metabolite phytoalexin are markedly induced in antagonist *Candida oleophila*-treated grapefruit (Droby et al., 2002). Interestingly, we have previously found some secondary metabolite-related enzyme activities that were significantly enhanced in response to yeast antagonist *Rhodosporidium paludigenum* Fell & Tallman application (Lu et al., 2013). The accumulation of several phenolic compounds, as well as coumarin scoparone, citrusnin A, and drupanin aldehyde, was recently observed in the metabolic profile of resistance-elicited citrus fruit (Ballester, Lafuente, de Vos, Bovy, & González-Candelas, 2013).

In our previous studies, *R. paludigenum* significantly inhibited various fungal diseases via induced disease resistance, and was more efficient than the other antagonistic yeasts investigated (Lu et al., 2013). However, the mechanisms by which *R. paludigenum* inhibits various postharvest diseases beyond the increased defense-related enzymes remain to be clarified, and processes are believed to be dependent on various host activities rather than a single one. In this study, we addressed this issue using an Affymetrix subgenomic oligoarray containing 30,279 independently expressed sequence tags (ESTs) from various Citrus species. This method provides the advantage of detecting global changes in fruit peel transcriptome profiling following the application of antagonistic yeast, which is a powerful tool that can be used to elucidate the mechanisms that contribute to resistance. In addition, the regulation of host-related defense gene ontology (GO) terms and pathway in the surface wound for yeast application are presented.

2. Materials and methods

2.1. Plant material and biocontrol agent

Mandarin orange (*Citrus reticulata* Blanco cv. Ponkan) fruit used throughout this study were harvested from a local orchard located at Chun'an city (Zhejiang Province, China) and immediately used after harvest. Fruit without physical injuries or infections were surface-sterilized by dipping in 0.1% sodium hypochlorite for 2 min, extensively washed with tap water and allowed to air dry at room temperature (20 °C).

An originally isolated yeast *R. paludigenum* Fell & Tallman identified by CABI Bioscience Identification Services (IMI 394084) was used in this experiment. The yeast was grown under agitation (200 rpm) on a controlled environment incubator shaker in 250-ml Erlenmeyer flasks containing 50 ml of nutrient yeast dextrose broth medium (NYDB, containing 8 g nutrient broth, 5 g yeast extract, and 10 g glucose in 1 l of distilled water) for 36 h at 28 °C in the dark. Yeast culture was then centrifuged (KA1000, Shanghai Anke, Shanghai, China) at 4000 rpm for 15 min. Yeast cells were resuspended twice in sterile, high purity water. The final cell suspension concentration was adjusted to 1×10^8 cells/ml with a hemocytometer.

2.2. Fruit inoculation

Surface-sterilized mandarin orange fruit was gently wounded (2-mm-deep and 5-mm-wide) with a sterile borer at two sites around blossom end. Fifty microlitres of either a yeast cell suspension of *R. paludigenum* (1×10^8 cells/ml) or sterile distilled water (control) were pipetted into each wound and allowed to dry at room temperature. Then, the fruit was stored in enclosed plastic trays to maintain high (90–95%) relative humidity at 25 °C. Wound sites were removed using a sterile borer under aseptic conditions after 0, 24, 48, 72 and 96 h of incubation and immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation and phenolic acid extraction.

2.3. RNA extraction and expression microarray hybridization

Total RNA was isolated from frozen tissue as described by Ballester, Lafuente, and González-Candelas (2006) with some modifications. First-strand cDNA was synthesized with a PrimeScript® RT reagent Kit with gDNA Eraser (RR047A, TaKaRa) from 1 μg of Total RNA according to the manufacturer's instructions. Global gene expression in mandarin orange was analysed using an Affymetrix subgenomic array containing 30,279 ESTs from various Citrus species (Affymetrix, Santa Clara, CA, USA). The arrays were hybridized and scanned at high resolution on the GeneChip Scanner 3000 system located at CapitalBio (Bioassay Laboratory of CapitalBio Corporation, Changping District, Beijing, China). The results were normalised and filtered using the GeneSpring GX 12.1 software (Silicon Genetics, Redwood City, CA, USA) with Affymetrix citrus technology. Differentially expressed genes with fold change (FC) greater than or equal to 2 (up or down) were considered for the present study.

The annotation of all ESTs from the microarray was updated by comparison (HarvEST: Citrus, http://harvest.ucr.edu/; *E* value < 10^{-10}) with the Arabidopsis Information Resource (http://www.arabidopsis.org/) database, and functionally classified using the Protein Analysis Through Evolutionary Relationships (PANTHER) classification system with default settings (Thomas et al., 2003). AgriGO and REViGO software (Du, Zhou, Ling, Zhang, & Su, 2010; Supek, Bosnjak, Skunca, & Smuc, 2011) were used for GO annotation and enrichment analysis. GO enrichment analysis for each expression pattern was calculated by a Yekutieli test model with false discovery rate of less than 0.05. The PathExpress (Goffard & Weiller, 2007) program was used to identify significantly overrepresented metabolic pathways using the default parameters (*P*-value ⩽0.01). Visualisation of biotic stress was displayed using MapMan software (Thimm et al., 2004).

2.4. Reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR)

For quantification of the mRNAs accumulated in the surface-wound, RT-qPCR analysis was performed with iTaq™ SYBR® Green Supermix With ROX (172-5851, Bio-Rad, USA) using the StepOne Real-time PCR instrument (v. 2.2.2, Applied Biosystems). The gene-specific primers were designed with the Primer Premier 5.0 program (PREMIER Biosoft International, Palo Alto, CA, USA) and are listed in Supplementary file 1. Primers were used at 0.2 μM each with the equivalent of 50 ng reverse-transcribed RNA template per reaction. The thermal cycling conditions were 95 °C for 3 min; followed by 40 cycles at 95 °C for 15 s, and then 60 °C for 45 s. The melt curve conditions were 95 °C 15 s, 60–95 °C by 0.3 °C steps and 95 °C 15 s. Fluorescent signals were collected during the annealing and extension cycle. Cycle threshold (*CT*) values were determined using autothreshold. For each sample, the relative expression (ΔCt) value for the gene of interest was obtained by normalising to *ubq11* transcript frequency. $\Delta\Delta Ct$ was calculated by subtracting ΔCt of controls replicate with the value from the ΔCt of all individual replications at the same time point. The relative mRNA expression was derived by the formula 2 [exp $(-\Delta\Delta Ct)$].

2.5. Determination of phenolic acids and lignin production in mandarin orange peel

2.5.1. Isolation of phenolic acids

Phenolic acids were isolated from extracts according to Xu, Ye, Chen, and Liu (2007) described methods with certain modifications. Mandarin orange peel powder (2 g) was extracted three times with 50 ml of 80% aqueous methanol and assisted by ultrasonic for 30 min at room temperature. Then it was centrifuged at 5000 rpm for 10 min. The combined supernatant was evaporated under vacuum at 40 °C until approximately 20 ml of filtrate remained. The residue was hydrolysed with 10 ml of 4 M NaOH for 4 h at room temperature and then acidified to pH 2 with 6 M HCl. The solution acidified was extracted three times with 20 ml of diethyl ether/ethyl acetate (1:1, v/v). The clear organic phase was combined and concentrated to dryness in a rotary vacuum evaporator at 35 °C, and 80% methanol was added to make a final volume of 10 ml. The combined methanol solution at constant volume of 35 ml was filtered through a 0.45 μm microporous membrane and used for the identification and quantification of the total phenolic acids.

2.5.2. HPLC analysis

Phenolic acids of HPLC analyses were carried out on an Alliance 2695 separations module (Waters) linked simultaneously to a PDA 2996 (Waters). The prepared phenolic acid solution was filtered through a Millipore membrane (0.45 μm) before injection, and 10 μl was injected on the reversed phase column (250 × 4.6 mm i.d.). The column thermostat was set at 40 °C. Elution was performed isocratically with the mobile phase consisting of 4% (v/v) acetic acid in water/100% methanol (80:20, v/v) at a solvent flow rate of 1 ml/min, which was the method of Subba Rao and Muralikrishna (2002), used with minor revision. After each run, the column was washed with 100% methanol and equilibrated to initial conditions for 15 min. Chromatograms were recorded at 320 nm for caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acid. Phenolic acids were identified by the retention time and the UV–vis spectra of standards. Quantification of phenolic acids was carried out by an external standard method using calibration curves, and concentration of phenolic acids was expressed as micrograms per gram of dry weight (mg/g DW).

2.5.3. Quantification of lignin

Lignin was assayed by derivatization with thioglycolic acid (modified from the method of Bruce & West, 1989). Peel tissues (1.2 g) of each treatment were firstly homogenated in 6 ml of methanol. Pellets were collected by centrifugation and transferred to glass beakers for drying (24 h at 45 °C). The resulting residue (approximately 10–15 mg) was transferred to glass screw-cap tubes, and then 1 ml of 2 M HCl and 0.2 ml of thioglycolic acid (T3758, Aldrich) were added. The tubes were sealed and incubated at 100 °C for 12 h to hydrate the methanol insoluble residue. After cooling to room temperature, the mixture was centrifuged at 12,000 rpm for 30 min at 4 °C. The collected pellet was washed once with 1 ml of distilled water, suspended in 1 ml of 0.5 M NaOH, and vigorously shaken for 18 h at 25 °C to extract the lignin thioglycolic acid. Following centrifugation as above, the supernatant was decanted into a glass centrifuge tube, and the pellet was washed with 0.5 ml of 0.5 M NaOH. The combined alkali extract was acidified with 1 ml of concentrated HCl and the lignin thioglycolic acid was allowed to precipitate at 4 °C for 6 h. The mixture was centrifuged as above again, and the final pellet was dissolved in 1 ml of 0.5 M NaOH. Absorption at 280 nm was then measured and the calibration curve was obtained using a alkalilignin (471003, Aldrich). Results were expressed as μg lignin (alkali) per mg dry weight tissue residue.

2.6. Statistical analyses

Phenolic acids and lignin contents were analysed with the Statistical Program SPSS/PC ver. II. x (SPSS Inc. Chicago, Illinois, USA). The values were the means of three replicate samples ± standard deviation (SD) and analysed at each time point according to the Student's *t*-test. Mean differences at $P < 0.05$ were considered to be significant.

3. Results

3.1. Regulation of gene expression changes in the response of mandarin orange to R. paludigenum induction

To investigate the molecular responses to biocontrol yeast treatment in mandarin orange, a transcriptome analysis approach was utilised. The scattplot in Supplementary file 2A depicts the changes in genes with altered expression patterns that were identified in two independent microarray experiments. Data analysis revealed 1666 probe sets corresponding to 713 differentially expressed unigenes, which showed a significant two-fold change in expression. Around 63% (1055 probes) of these probes were up-regulated in the treated samples compared to the controls, and 37% (611 probes) were down-regulated. The multi-sample histogram in Supplementary file 2B reveals evident differences in the normalised intensity values between the yeast-treated groups and control groups. These results demonstrate that the intensity value in the yeast-treated group was significantly more positive, ranging from 1.67 to 3.34, than that in the control.

Amongst the differentially expressed genes, 72.3% had an assigned *Arabidopsis thaliana* homologue candidate, and functionally classified into several major functional categories according to their putative function (Fig. 1). As illustrated in Fig. 1, the most abundant functional category amongst the differentially expressed genes was metabolic process (35.69%), followed by cellular process (12.91%), immune system process (11.07%), and transport (8.74%). Only one up-regulated genes fell into the homeostatic process. More down-regulated genes were categorised into the reproduction process than up-regulated genes. Moreover, cell communication (8.50%), immune system process (11.07%), and response to

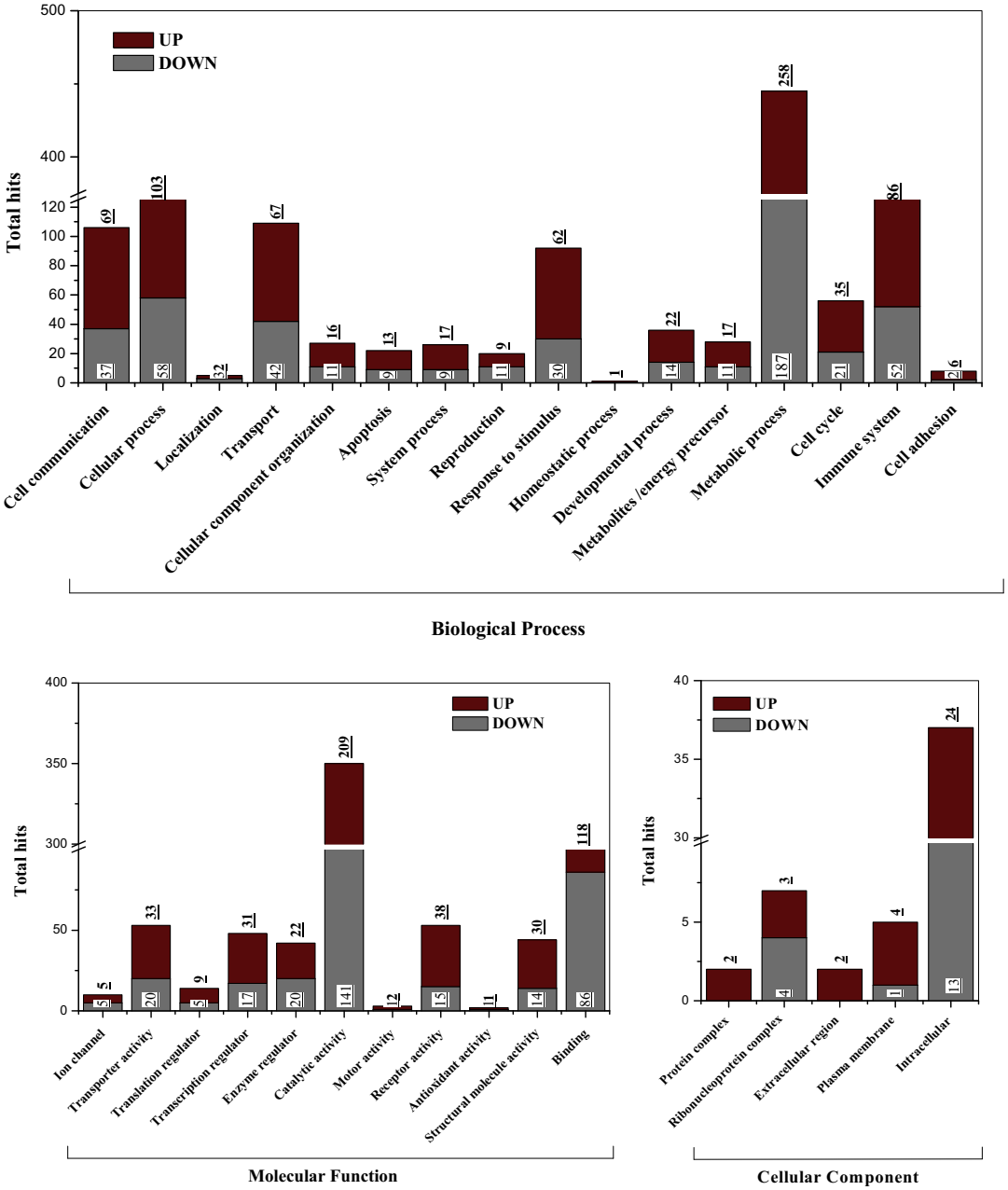


Fig. 1. Gene ontology classification of the differentially expressed unigenes in the *Rhodosporidium paludigenum* treated mandarin orange fruit with the PANTHER classification system. Seventy-two point three percent of the difference expressed genes had an assigned *Arabidopsis thaliana* homologue candidate, and functional classified into several major functional categories according to their putative function.

stimulus (7.38%), which are associated with the plant defense system, had more up-regulated than down-regulated genes.

3.2. Transcriptome profiling in the response of mandarin orange to R. paludigenum induction

Significant transcriptional changes in response to biocontrol agent *R. paludigenum* were also observed in a group of genes involved in hormone biosynthesis, especially those related to ethylene and JA synthesis (Table 1). An increase in the expression of the genes encoding for S-adenosyl-L-methionine (*SAM*), 1-aminocyclopropane-1-carboxylate synthase (*ACS*), 1-aminocyclopropane-1-carboxylic acid oxidase (*ACO*), and methionine adenosyltransferase (*MTO*) was observed in this study. The expression of these four genes was involved in the biosynthesis of ethylene. The expression of 12-oxophytodienoate reductase (*OPR1*) and coronatine insensitive 1 (*COI 1*), which are two genes related to JA induction, also increased following *R. paludigenum* application. Auxin is also an essential factor in transducing signals generated in response to disease resistance induction. Amongst them, transcripts related to *GH3*-like proteins involved in auxin synthesis (*GH3.1*, *WES1*, *GH3.4*, and Aldo-keto reductase) were more abundant in yeast-treated fruit. By contrast, transcript abundance of lipoxigenase2 (*LOX2*), which is involved in jasmonate biosynthesis, was low in the fruit following *R. paludigenum* application. Several genes involved in ethylene response and GA synthesis were also down-regulated, including ethylene-responsive element-binding factor (*ATERF13*), ethylene-responsive factor (*ERF1*), ethylene-responsive family protein, and gibberellin 2-oxidase (*ATGA2OX8*).

Table 1
Citrus genes related to phytohormone metabolism with *Arabidopsis thaliana* homologies in antagonistic yeast *Rhodosporidium paludigenum* elicited mandarin oranges based on microarray analyses in MapMan software (fold changes, FC > 2).

Probe ID	TAIR ID	*E*-value	Symbol	Description	Ratio (log_2FC)
Ethylene metabolism					
Cit.12979.1.S1_at	AT5G66430.1	2e-63	*SAM*	S-adenosyl-L-methionine	3.57
Cit.4491.1.s1_at	AT4G11280.1	0.0	*ACS6*	1-aminocyclopropane-1-carboxylate synthase	1.66
Cit.1718.1.s1_s_at	AT2G19590.1	1e-127	*ACO1*	1-aminocyclopropane-1-carboxylate oxidase	1.88
Cit.25886.1.S1_at	AT3G17390.1	1e-88	MTO3	Methionine adenosyltransferase	1.41
Cit.29675.1.s1_s_at	AT2G44840.1	4e-33	*ATERF13*	Ethylene-responsive element binding factor	−1.17
Cit.34546.1.S1_at	AT3G23240.1	1e-10	*ERF1*	Ethylene-responsive factor 1	−2.97
Cit.30665.1.s1_s_at	AT4G29100.1	9e-92	–	Ethylene-responsive family protein	−1.47
Cit.14303.1.s1_at	AT5G61590.1	3e-37	–	AP2 domain-containing transcription factor	−1.56
Jasmonic acid metabolism					
Cit.30339.1.s1_s_at	AT1G76680.1	1e-121	*OPR1*	12-oxophytodienoate reductase	1.83
Cit.3644.1.S1_at	AT2G39940.1	3e-55	*COI1*	Coronatine insensitive 1	1.12
Cit.22219.1.s1_s_at	AT3G45140.1	0.0	*LOX2*	Lipoxygenase	−1.17
Auxin metabolism					
Cit.5201.1.s1_at	AT2G14960.1	0	*GH3.1*	GH3.1	2.87
Cit.26243.1.s1_at	AT4G27260.1	2e-42	*WES1*	Indole-3-acetic acid amido synthetase	1.05
Cit.7548.1.s1_at	AT1G59500.1	3e-46	*GH3.4*	Indole-3-acetic acid amido synthetase	2.61
Cit.23626.1.s1_x_at	AT1G60680.1	4e-58	*AGD2*	Aldo-keto reductase	4.15
Gibberellins metabolism					
Cit.26276.1.s1_at	AT4G21200.1	2e-22	*ATGA2ox8*	Gibberellins 2-oxidase 8	−1.60
Cit.30950.1.s1_at	AT2G30810.1	5e-33	–	Gibberellins-regulated family protein	−1.04

Several genes involved in defense-related proteins and secondary metabolism were differentially expressed following *R. paludigenum* application, including those involved in the biosynthesis of *PR* proteins, phenylpropanoids, dihydroflavonols, lignin, and lignans. A significant increase in the expression of the genes encoding for *beta*-1,3-glucanase, CHI, chitin and lipid binding protein, PAL, 4-coumarate-CoA ligase (*4CL3*), quinate *O*-hydroxycinnamoyltransferase (*HCT*), cinnamoyl-CoA reductase-related protein, and caffeate O-methyltransferase (*ATOMT1*) was observed (Table 2). However, genes involved in the biosynthesis of thaumatin and other secondary metabolites, such as chalcones, isoflavonols, and betaines, were down-regulated (Table 2), as proven by

Table 2
Citrus genes related to defense-related proteins and secondary metabolism with *Arabidopsis thaliana* homologies in antagonistic yeast *Rhodosporidium paludigenum* elicited mandarin oranges based on microarray analyses in MapMan software (fold changes, FC > 2).

Probe ID	TAIR ID	*E*-value	Symbol	Description	Ratio (log_2FC)
Defence-related proteins and response to stress					
Cit.8584.1.s1_s_at	AT2G14580.1	8e-56	*ATPRB1*	Basic pathogenesis-related protein 1	2.08
Cit.9704.1.S1_at	AT3G57270.1	1e-105	*BG1*	Beta-1,3-glucanase 1	1.18
Cit.21778.1.S1_at	AT3G57240.1	9e-13	*BG3*	Beta-1,3-glucanase 3	1.09
Cit.22311.1.s1_s_at	AT5G24090.1	1e-126	*CHIB1*	Acidic endochitinase	1.09
Cit.1727.1.s1_s_at	AT3G54420.1	6e-76	*CHI*	Chitinase (*PR3*)	1.07
Cit.580.1.s1_x_at	AT3G04720.1	9e-47	*PR4*	Chitin binding	1.33
Cit.1201.1.s1_at	AT4G11650.1	9e-81	*ATOSM34*	Osmotin 34 (*PR5-1*)	1.53
Cit.4233.1.s1_s_at	AT1G74190.1	6e-50	*AtRLP15*	Protein binding	1.17
Cit.10753.1.s1_at	AT4G19040.2	1e-60	*EDR2*	Lipid binding	1.22
Cit.30907.1.s1_at	AT4G19800.1	7e-62	–	Glycosyl hydrolase family 18 protein	1.35
Cit.30775.1.s1_s_at	AT4G38660.1	1e-100	*Thaumatin*	Thaumatin, putative	−1.10
Cit.31825.1.s1_at	AT4G25780.1	4e-31	–	Pathogenesis-related protein	−1.74
Secondary metabolism					
Cit.24218.1.s1_s_at	AT2G37040.1	0.0	*PAL1*	Phenylalanine ammonia-lyase	1.07
Cit.11987.1.s1_s_at	AT1G65060.1	0.0	*4CL3*	4-coumarate-CoA ligase	1.11
Cit.17520.1.S1_at	AT2G30490.1	0.0	*C4H*	Trans-cinnamate 4-monooxygenase	1.41
Cit.14337.1.s1_at	AT5G48930.1	4e-49	*HCT*	Quinate O-hydroxycinnamoyltransferase	2.63
Cit.6310.1.s1_at	AT4G35160.1	4e-52	–	O-methyltransferase family 2 protein	4.35
Cit.28630.1.s1_at	AT3G02280.1	1e-47	–	Flavodoxin family protein	1.26
Cit.11230.1.s1_at	AT1G28680.1	2e-77	–	Transferase family protein	1.25
Cit.13313.1.s1_s_at	AT5G14700.1	1e-105	–	Cinnamoyl-CoA reductase-related	1.07
Cit.4292.1.s1_at	AT5G54160.1	1e-113	*ATOMT*	Caffeate O-methyltransferase	3.09
Cit.40492.1.s1_s_at	AT5G54010.1	3e-95	–	Glycosyltransferase family protein	3.84
Cit.32640.1.s1_at	AT5G54000.1	5e-55	–	Oxidoreductase	1.40
Cit.30441.1.s1_at	AT3G16640.1	6e-49	*TCTP*	Translationally controlled tumour protein	4.12
Cit.16670.1.s1_at	AT4G36810.1	2e-79	*GGPS1*	Geranyleranyl pyrophosphate synthase 1	1.12
Cit.16301.1.s1_at	AT2G20340.1	2e-63	–	Tyrosine decarboxylase, putative	2.22
Cit.30505.1.s1_at	AT5G07130.1	1e-53	*LAC13*	Laccase 13	1.32
Cit.37373.1.s1_at	AT2G18570.1	9e-37	–	UDP-glucoronosyl	−3.06
Cit.10247.1.s1_at	AT4G35150.1	9e-30	–	O-methyltransferase family 2 protein	−3.76
Cit.30458.1.s1_s_at	AT5G13930.1	0.0	*CHS*	Naringenin-chalcone synthase	−2.62
Cit.22506.1.s1_at	AT4G39230.1	7e-44	–	Isoflavone reductase, putative	−1.38
Cit.5103.1.s1_at	AT3G51420.1	1e-50	–	Strictosidine synthase family protein	−1.76
Cit.30486.1.s1_s_at	AT3G48000.1	0.0	*ALDH2*	Aldehyde dehydrogenase 2	−1.55

a strong decrease in the expression of genes encoding for naringenin–chalcone synthase, isoflavone reductase, and aldehyde dehydrogenase.

3.3. Functional analysis based on the transcriptome profiles

To evaluate the potential functions of genes enriched in mandarin orange peel tissue in response to *R. paludigenum* application, microarray data was assigned to the GO category of biological processes. The GOs were then compared by semantic similarity-based scatter plots using REViGO (Fig. 2). This method removes redundant GO terms that are significantly up-regulated and down-regulated in each main gene category by the biocontrol yeast. REViGO data analyses revealed that up-regulated GO terms were involved in the biotic stress stimulus response (defense response to fungus, response to biotic stimulus, and regulation of response to stress), secondary metabolism (phenylalanine biosynthesis, coumarin biosynthesis, tyrosine metabolism, alkene biosynthesis, and aromatic compound biosynthesis), polysaccharide metabolism (chitin catabolism and chitin metabolism), and glycoside metabolism (Fig. 2A). Meanwhile, functional classes with down-regulated gene expression included saccharine metabolism, starch metabolism, and gametophyte development (Fig. 2B).

The second enrichment method was conducted using the Path-Express program (Goffard & Weiller, 2007) to determine which metabolic pathways were significantly ($P \leq 0.01$) over-represented

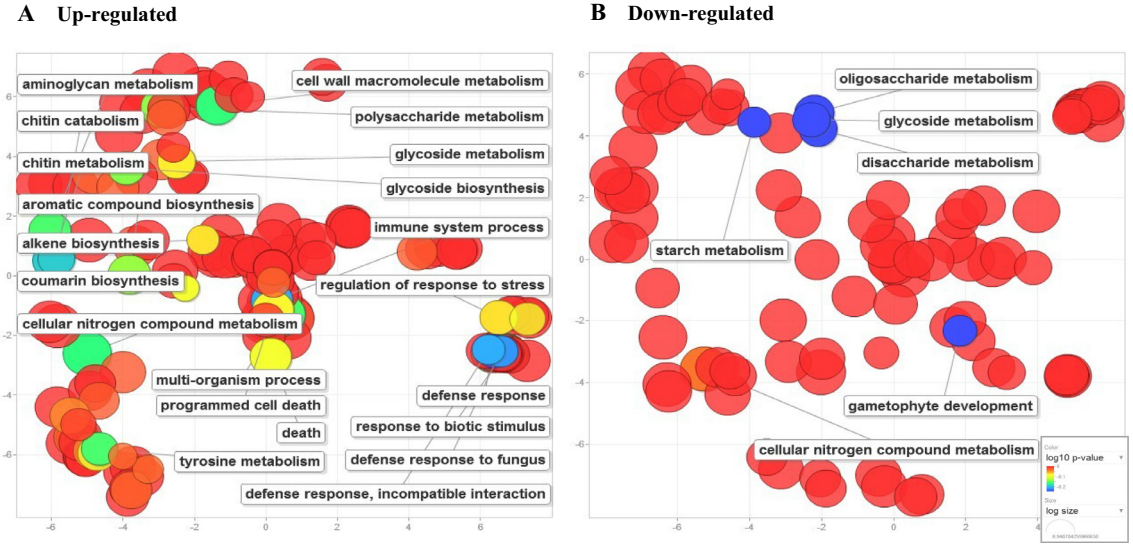


Fig. 2. The summarisation of non-redundant gene ontology (GO) terms in mandarin orange fruit following interaction with antagonistic yeast *Rhodosporidium paludigenum*. The up-regulated GO terms are shown in part (A), and the down-regulated GO terms are shown in part (B).

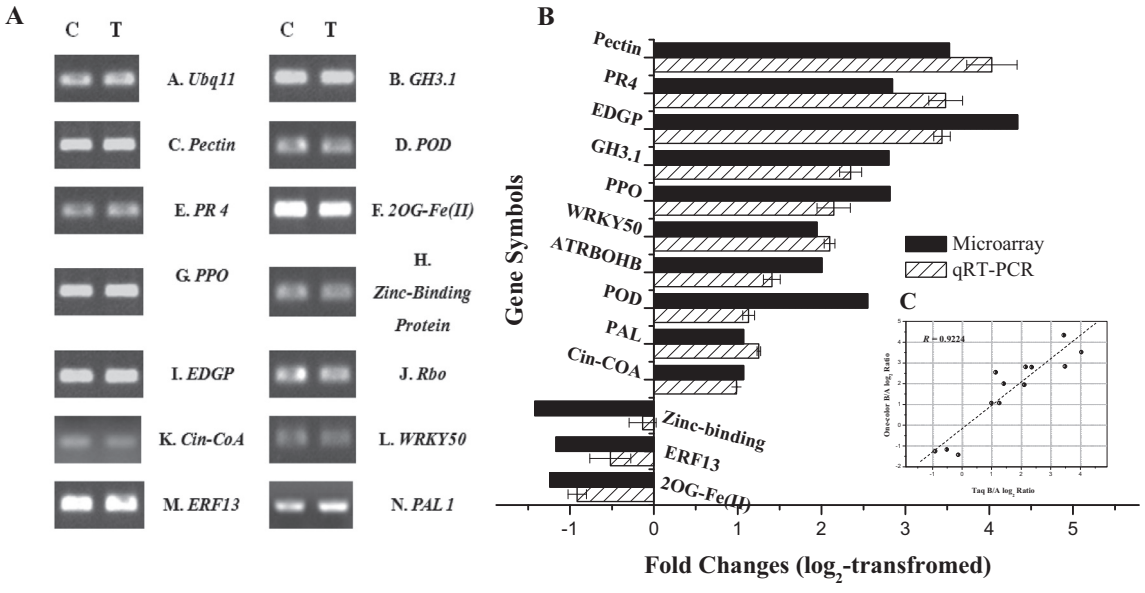


Fig. 3. The differentially expressed genes profile detected by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) of *Ubq11* (A), *GH3.1* (B), pectinesterase family protein (C), peroxidase (D), pathogenesis-related protein 4 (E), 2OG-Fe(II) oxygenase family protein (F), polyphenol oxidase (G), zinc-binding dehydrogenase family protein (H), *EDGP* (I), respiratory burst oxidase homologue B (*rbo*) (J), cinnamoyl-CoA reductase (K), *WRKY50* (L), ethylene responsive element binding factor 13 (M), and phenylalanine ammonia-lyase (N).

following *R. paludigenum* application (Supplementary file 3). PathExpress uses the subset of submitted genes that can be assigned with EC numbers, and reports all metabolic networks that include these EC numbers and enzymes in these networks corresponding to submitted identifiers. This analysis showed that genes induced in yeast-treated fruits, compared with those in untreated fruits, were mainly involved in pathways associated with secondary metabolism (phenylalanine metabolism, phenylpropanoid biosynthesis, tyrosine metabolism, and alkaloid biosynthesis I), particularly that of phenylpropanoids. Analysis using PathExpress identified starch and sucrose metabolism as being the only significantly down-regulated pathways.

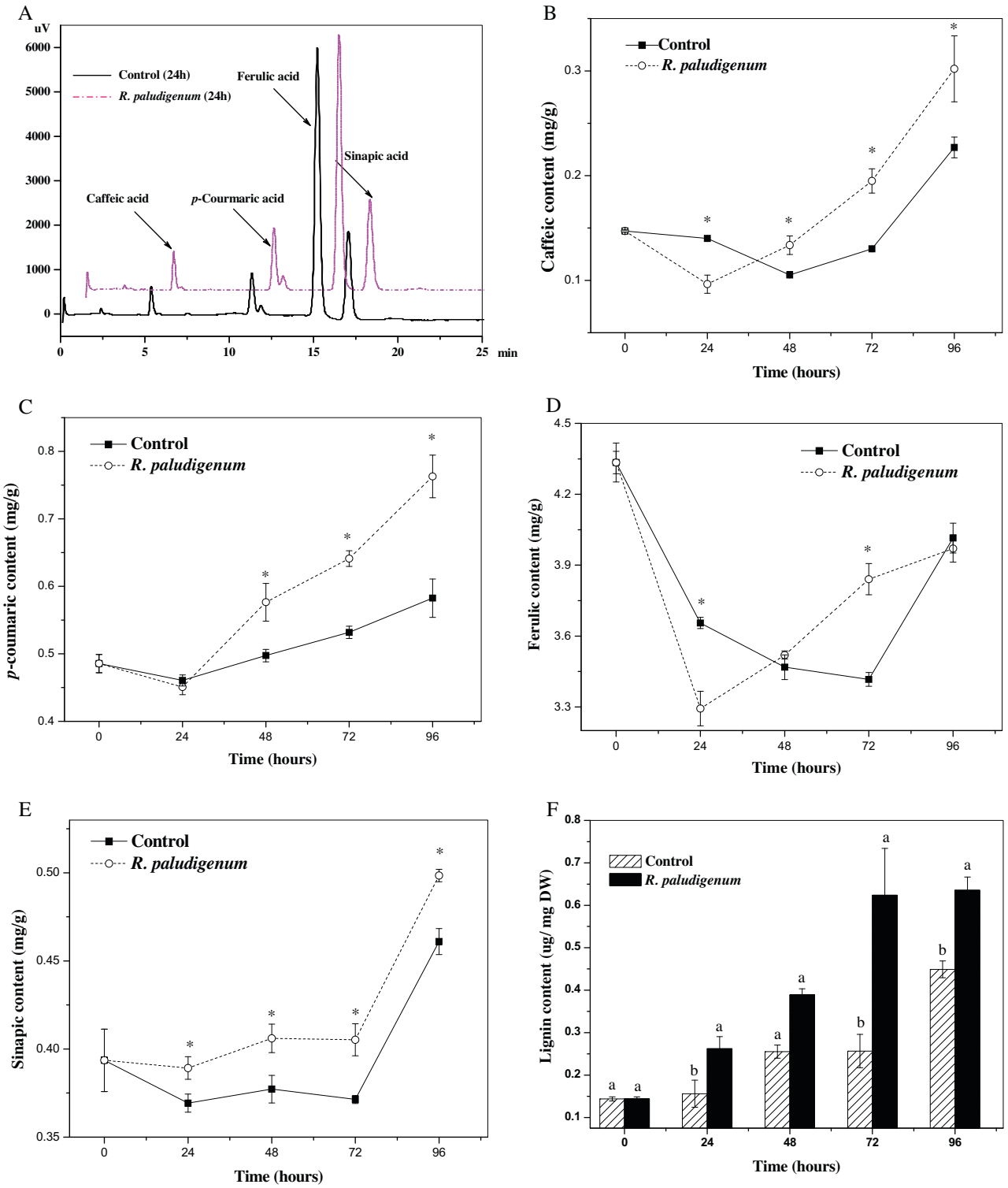


Fig. 4. Effect of *Rhodosporidium paludigenum* on the level of four phenolic acids (A), including caffeic acids (B), *p*-coumaric acids (C), ferulic acids (D) and sinapic acids (E), and lignin content (F) in mandarin orange fruit. The fruit were kept for various time intervals (0, 24, 48, 72 and 96 h) after antagonistic yeast treatment at 25 °C and 90–95% RH, before phenolic extracts were obtained. Short bars represent standard errors of three replicates. Asterisks and different letters indicate significant differences for decay ($P \leq 0.05$) according to Student's t-test at each time point.

3.4. Verification of gene expression pattern with reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR)

Transcriptional regulation revealed by microarray data was confirmed by RT-qPCR analysis of mRNA expression. Fourteen genes associated with oxidative stress, hormone metabolism, and defense responses were selected for verification (Fig. 3A). Amongst the oxidative stress-related transcripts, peroxidase and respiratory burst oxidase (*Rbo*) were expressed higher in yeast-treated fruits. The transcript abundance of chitin-binding protein (*PR4*), extracellular dermal glycoprotein (*EDGP*), *PAL1*, cinnamoyl-CoA reductase, polyphenol oxidase, and transcription factor WRKY50, which are all involved in plant defense responses, was higher in yeast-treated fruit than that in the untreated control (Fig. 3B). The results of RT-qPCR analysis show good correlation with the expression profiling obtained from the microarray hybridization experiments ($R = 0.9224$, Fig. 3C). Thus, the increased and decreased expression patterns of microarray analysis were supported by RT-qPCR analyses.

3.5. Phenolic acid and lignin production level in mandarin orange following R. paludigenum application

The biosynthesis of phenylpropanoids and compounds derived from this pathway are important in the resistance induction of mandarin orange against biotic and abiotic stresses. To validate the up-regulated genes and pathways involved in secondary metabolism, the concentrations of four types of phenolic acids (caffeic, *p*-coumaric, ferulic, and sinapic acids) in mandarin orange peel were measured (Fig. 4A). The time points included in the analysis were 0, 24, 48, 72, and 96 h after yeast treatment. The results show that *p*-coumaric acid content in the peel tissue extracts was significantly higher after 48, 72, and 96 h following yeast application compared with that in the water control (Fig. 4C). However, caffeic and ferulic acids were lower at 24 h in yeast-treated samples than those in the control (Fig. 4B and D). *R. paludigenum* significantly induced the production of caffeic and ferulic acids in the next 48 and 24 h, respectively. Meanwhile, the amount of sinapic acid was higher in yeast-treated wounds in all time points (except 0 h) compared with that in the control wounds (Fig. 4E). Besides phenolic acids, the lignin production level in mandarin orange peel is shown in Fig. 4F. As with the change in the sinapic acid content, the lignin production level was higher in yeast-treated wounds at three time points (24, 72, and 96 h) than that in the control wounds.

4. Discussion

4.1. General

Active disease resistance in fruits is widely recognised as part of the complex disease reduction mechanism of biocontrol agents, and increases protection of fruits against virulent pathogens (de Capdeville, Wilson, Beer, & Aist, 2002). However, our knowledge of the process underlying the establishment of disease resistance induction in fruits is still poor and limited to single metabolites, enzymes, or genes. In this study, high-throughput technologies and genome-wide analysis approach were used to reveal the genes and biological pathways enriched in mandarin orange in response to antagonistic yeast *R. paludigenum* interaction. Our result showed that *R. paludigenum* treatment led to substantial changes (greater than or equal to 2-fold) of mRNAs relative to control with water, including 1055 up-regulated and 611 down-regulated genes. Functional categorisation of the differentially expressed genes revealed that signalling pathway, cell development, immune system, stress and defense responses were the most differentially regulated processes occurring during fruit resistance induction.

4.2. Functions of the hormones in the response of mandarin orange to R. paludigenum induction

Ethylene and JA, two major plant hormones synthesized from methionine and α-linolenic acid in most tissues, are the important signals in response to stress through regulation of many other defense-related genes against necrotrophic pathogens in plants (Kunkel & Brooks, 2002). Such genes include hevein-like protein *HEL*, basic chitinase *CHIB*, plant defensin *PDF 1.2* in *Arabidopsis* (Norman-Setterblad, Vidal, & Palva, 2000), and *PAL*, acidic class II chitinase in citrus fruit (Marcos, Gonzalez-Candelas, & Zacarias, 2005). In the present study, a coordinated increase in four ethylene related genes *SAM*, *ACS6*, *ACO1*, and *MTO3* mRNA expression was clearly observed in *R. paludigenum*-treated mandarin orange transcriptome (Table 1). The gene expression levels of *SAM, ACS6, ACO1*, and *MTO3* increased by 257%, 66%, 88% and 41% in these antagonistic yeast treatment groups, respectively. Importantly, two genes *ACS* and *ACO*, amongst these up-regulated key ethylene synthesis genes, were regarded as the key limiting factors in ethylene biosynthesis, indicating an involvement of plant hormone ethylene in inducible resistance induction by microbes in fruits. Moreover, this finding also suggested that ethylene-mediated defense responses may also involve in improving host protection under biotic stress.

Similarly, an obvious increase of two genes associated with JA biosynthesis (*OPR1*) and response (*COI1*) was detected in *R. paludigenum* treated peel tissue. Meanwhile, one JA biosynthesis key gene *LOX2* was down-regulated after strain inoculation (Table 1). Limited information about the functions of JA in the resistance mechanisms of fruits is available, but the up-regulation of *COI1*, which is required in most JA-modulated secondary metabolism, defense, and hormone interactions (Devoto et al., 2005), confirmed that the fruits could also increase their protection via enhancing their perception to JA signal in response to biotic stress stimuli.

The auxin IAA is the earliest identified and most important auxin in higher plants. Previous studies have revealed its physiological regulatory activity in delaying the ripening and softening of fruits, thereby inhibiting postharvest physiological disorders and inducing defense responses in plant tissues (Mayda, Marques, Conejero, & Vera, 2000). Here, three IAA amido synthetase-related genes (*WES1*, *GH3.4*, and *AGD2*) and one auxin response gene (*GH3.1*) were also markedly induced by *R. paludigenum* (Table 1), suggesting that the presence of antagonistic yeast increased the production of IAA in host tissue. In addition, this observation was the direct evidence proving that hormone IAA and auxin were involved in biocontrol agent-mediated orange fruit disease resistance.

In a plant, defense responses are not only modulated by individual hormones, but also by the crosstalk between various hormone-response pathways (Castoria & Wright, 2010). Ethylene, auxin, and GA pathways are considered as hormone modulators of the SA–JA signalling backbone; Auxin and JA signals were reported to repress SA levels and signalling, and GH3s act as regulatory nodes in modulating hormone crosstalk (Robert-Seilaniantz, Grant, & Jones, 2011). This so-called hormone crosstalk can provide the plant with a powerful capacity to finely modulate its immune response with the invader encountered, and utilise its resources in a cost-efficient manner (Pieterse et al., 2012). In Table 1, a large amount of genes associated with ethylene, auxin, IAA and GA were observed in the present study. Meanwhile, no significant change in the SA biosynthesis-related gene was detected, unsurpringly suggesting that signalling communication, especially interaction between plant hormones, also existed in postharvest fruit.

4.3. Involvement of the defense-related proteins and secondary metabolites in the response of mandarin orange to R. paludigenum induction

Plants use an intricate defense system against pests and pathogens, including the accumulation of various types of stress proteins with putative protective functions, production of low molecular mass secondary metabolites with antimicrobial activity, and enhancement of structural defensive barriers, such as lignin biosynthesis. PR proteins are one of these plant protective functions induced only in pathological or related situations, and are suspected to promote protection against fungi through hydrolytic activities on cell walls, contact toxicity, or possibly an involvement in defense signalling (van Loon, Rep, & Pieterse, 2006). Data from the present study show a clear induction of *BG1*, *BG3*, *CHIB1*, *CHI*, and *PR4* mRNA in *R. paludigenum*-treated fruits (Table 2), and verified the contributions of two hydrolytic proteins in the induction of fruit resistance. This observation agreed with a previous report that showed a transient increase in PR-2 (β-1,3-glucanases) and PR-3 (*CHI*) activities in *A. pullulans*-incubated apple fruit (Ippolito, El Ghaouth, Wilson, & Wisniewski, 2000). Our previous study confirmed this behaviour with a 1.25-fold increase in β-1,3-glucanase activity in *R. paludigenum*-treated mandarin orange fruits compared with the reference value (Lu et al., 2013). Meanwhile, basic *PR* protein 1 encoding genes, *PR5-1* (osmotin 34), and *PR-9* (peroxidases) were also up-regulated in antagonist-inoculated fruits (Table 2). PR-1, the dominant PR group induced by pathogens, SA, or ethylene, could enhance the resistance of tobacco plants against heavy metal and pathogen stresses, and is commonly used as a marker for systemic acquired resistance (Sarowar et al., 2005). The PR-9 family of peroxidases likely functions in strengthening plant cell walls by catalysing lignin deposition in reaction to a microbial attack.

Secondary metabolites are a class of naturally occurring compounds that are widely distributed in plants, most of which are regulated by plant hormones (Farmer, Alméras, & Krishnamurthy, 2003). Many secondary metabolites found in plants have a function in defense against pathogens, pests, and herbivores, and possess deterrence/anti-feedant activity or toxicity; they also act as precursors for physical defense systems (Bennett & Wallsgrove, 1994). The key branch point enzyme PAL catalyses the first step of the phenylpropanoid pathway, which leads to the production of phenolic compounds, and is considered to be activated by JA/ethylene signalling during plant defense responses. As expected in this study, the expression levels of nearly all the key genes involved in phenylpropanoid biosynthesis and metabolism (e.g., *PAL1*, *4CL3*, *C4H*, *HCT*, *ATOMT*, and cinnamoyl-CoA reductase) were up-regulated (Table 2). The pathway analysis results (Supplementary file 3) and phenolic acid HPLC detection results confirmed this observation (Fig. 4). A strong correlation was observed between the increased mRNA expression levels of phenolic acid-related synthesis genes (Table 2), and their increased content rates (Fig. 4B–E) in *R. paludigenum*-treated peel tissue from 24 h to 48 h after inoculation. The significance of this class of phenolics involved in defense responses against postharvest pathogen attack is well known, and this class of phenolics significantly affects host protection (Bennett & Wallsgrove, 1994). In the present study, the production of phenolic acid-derived compound lignin, which is a plant's major structural barrier against fungal invasion, showed a positive correlation with the rapidly induced *PAL* and other phenylpropanoid biosynthetic genes in fruit (Fig. 4F). These findings reveal that the enhancement of the structural defensive barriers of fruit peels and production of antimicrobial compounds were two of the resistance induction mechanisms for *R. paludigenum*.

Meanwhile, it is essential to underline that tyrosine metabolism and alkaloid biosynthesis were also supposed to contribute to disease resistance, because most of the known functions of alkaloids relate to protection. Furthermore, benzylisoquinoline alkaloids are the products from tyrosine through a sequence of enzymatic modifications (Bennett & Wallsgrove, 1994). In the current study, the up-regulation of tyrosine metabolism and alkaloid biosynthesis in the transcriptomic functional analysis was observed (Supplementary file 3), a finding similar to that of González-Candelas et al. (2010) who discovered a significant presence of isoprenoid, alkaloid and phenylpropanoid biosynthetic genes in the transcriptomic response of citrus fruits to *Penicillium digitatum* infection. Our results revealed that alkaloid and tyrosine metabolism played a positive role in fruit disease resistance induction against fungal pathogens and the changes of secondary metabolic were essentially involved in antagonistic microbes activated host protection in citrus fruit tissue.

4.4. Resource allocation and tradeoffs in R. paludigenum-inoculated orange fruit

Organisms are regarded as informed resource users that possess evolved priority systems for allocating limited resources and energy to various activities and structures in a hierarchical fashion (Rauw, 2012). When plants resist or mock-resist pathogen attack, limited resources and energy are diverted from growth and reproduction toward the defense reaction. In this study, a down-regulation of large amounts genes involved in starch and sucrose metabolism pathway, including starch metabolism, oligosaccharide and glycoside metabolism, was observed in antagonist-incubated orange fruits (Supplementary file 3). Meanwhile, a homeostatic process-related gene, which was supposed to orchestrate or coordinate changes in the metabolism of body tissues to support a certain physiological state, was significantly up-regulated (Fig. 1). Taking the up-regulated secondary metabolism into consideration, *R. paludigenum* was supposed to elicit an energy shift in metabolic priorities within the mandarin orange to those associated with immunity. This hypothesis was in agreement with Dietrich, Ploss, and Heil (2005), who found that the activation of direct defense reactions in *A. thaliana* against pathogens has a metabolic cost that reduces plant fitness.

However, for beneficial resistance and minimal resource waste, resistant plants should have greater fitness than non-resistant plants under diseased conditions. Upon treatment with antagonist elicitation, many plants develop an enhanced capacity to activate defense responses, which is called 'priming'. This phenomenon does not directly activate the plant's defense arsenal, but conditions the plant with a faster and stronger activation of stress-specific defense mechanisms against pathogen attack. This phenomenon is suggested to be a better strategy for plants and fruits that are avoiding these risks. Furthermore, it is meaningful to examine whether this phenomenon may contribute to *R. paludigenum*-induced disease resistance.

In conclusion, after an interaction with *R. paludigenum* which could increase disease resistance in mandarin orange, we observed a global effect on the transcriptional changes and several antifungal metabolites with increased abundance. Several biological processes associated with signal pathways, such as phytohormone biosynthesis, perception and even crosstalk, seemed to be affected, and many biotic stress-related protein biosyntheses and partials of secondary metabolism were up-regulated. On the other hand, a down-regulation of large amounts of genes involved in the starch and sucrose metabolism pathway revealed a possible energy shift in metabolic priorities within the mandarin orange to those associated with immunity. This discovery of inducible disease resistance induction in postharvest fruit by biocontrol yeast is of considerable interest and deserves much further study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.09.097.

References

Arras, G. (1996). Mode of action of an isolate of *Candida famata* in biological control of *Penicillium digitatum* in orange fruits. *Postharvest Biology and Technology, 8*, 191–198.

Ballester, A. R., Lafuente, M. T., de Vos, R. C. H., Bovy, A. G., & González-Candelas, L. (2013). Citrus phenylpropanoids and defence against pathogens. Part I: Metabolic profiling in elicited fruits. *Food Chemistry, 136*, 178–185.

Ballester, A. R., Lafuente, M. T., & González-Candelas, L. (2006). Spatial study of antioxidant enzymes, peroxidase and phenylalanine ammonia-lyase in the citrus fruit–*Penicillium digitatum* interaction. *Postharvest Biology and Technology, 39*, 115–124.

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytologist, 127*, 617–633.

Bostock, R. M. (2005). Signal crosstalk and induced resistance: Straddling the line between cost and benefit. *Annual Review of Phytopathology, 43*, 545–580.

Bruce, R. J., & West, C. A. (1989). Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. *Plant Physiology, 91*, 889–897.

Castoria, R., & Wright, S. (2010). Host responses to biological control agents postharvest pathology. *Postharvest Pathology*, 171–181.

Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature, 411*, 826–833.

de Capdeville, G., Wilson, C. L., Beer, S. V., & Aist, J. R. (2002). Alternative disease control agents induce resistance to blue mold in harvested 'red delicious' apple fruit. *Phytopathology, 92*, 900–908.

Devoto, A., Ellis, C., Magusin, A., Chang, H.-S., Chilcott, C., Zhu, T., et al. (2005). Expression profiling reveals *COI1* to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions. *Plant Molecular Biology, 58*, 497–513.

Dietrich, R., Ploss, K., & Heil, M. (2005). Growth responses and fitness costs after induction of pathogen resistance depend on environmental conditions. *Plant, Cell & Environment, 28*, 211–222.

Droby, S., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E. E., et al. (2002). Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*. *Phytopathology, 92*, 393–399.

Droby, S., Wisniewski, M., Macarisin, D., & Wilson, C. (2009). Twenty years of postharvest biocontrol research: Is it time for a new paradigm? *Postharvest Biology and Technology, 52*, 137–145.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., & Su, Z. (2010). AgriGO: A GO analysis toolkit for the agricultural community. *Nucleic Acids Research, 38*, W64–W70.

Farmer, E. E., Alméras, E., & Krishnamurthy, V. (2003). Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Current Opinion in Plant Biology, 6*, 372–378.

Fravel, D. R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology, 43*, 337–359.

Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., et al. (2006). Early signaling events induced by elicitors of plant defenses. *Molecular Plant–Microbe Interactions, 19*, 711–724.

Goffard, N., & Weiller, G. (2007). PathExpress: A web-based tool to identify relevant pathways in gene expression data. *Nucleic Acids Research, 35*, W176–W181.

González-Candelas, L., Alamar, S., Ballester, A. R., Sánchez-Torres, P., Forment, J., Gadea, J., et al. (2010). Global regulation of genes in citrus fruit in response to the postharvest pathogen *Penicillium digitatum*. In D. Prusky & M. L. Gullino (Eds.), *Postharvest pathology* (pp. 57–67). Netherlands: Academic Press, Springer.

Ippolito, A., El Ghaouth, A., Wilson, C. L., & Wisniewski, M. (2000). Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. *Postharvest Biology and Technology, 19*, 265–272.

Janisiewicz, W. J., & Korsten, L. (2002). Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology, 40*, 411–441.

Kunkel, B. N., & Brooks, D. M. (2002). Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology, 5*, 325–331.

Lu, L., Lu, H., Wu, C., Fang, W., Yu, C., Ye, C., et al. (2013). *Rhodosporidium paludigenum* induces resistance and defense-related responses against *Penicillium digitatum* in citrus fruit. *Postharvest Biology and Technology, 85*, 196–202.

Marcos, J. F., Gonzalez-Candelas, L., & Zacarias, L. (2005). Involvement of ethylene biosynthesis and perception in the susceptibility of citrus fruits to Penicillium digitatum infection and the accumulation of defence-related mRNAs. *Journal of Experimental Botany, 56*, 2183–2193.

Mayda, E., Marques, C., Conejero, V., & Vera, P. (2000). Expression of a pathogen-induced gene can be mimicked by auxin insensitivity. *Molecular Plant–Microbe Interactions, 13*, 23–31.

Norman-Setterblad, C., Vidal, S., & Palva, E. T. (2000). Interacting signal pathways control defense gene expression in Arabidopsis in response to cell wall-degrading enzymes from *Erwinia carotovora*. *Molecular Plant–Microbe Interactions, 13*, 430–438.

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology, 28*, 489–521.

Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics, 3*, 267.

Robert-Seilaniantz, A., Grant, M., & Jones, J. D. G. (2011). Hormone crosstalk in plant disease and defense: More than just JASMONATE–SALICYLATE antagonism. *Annual Review of Phytopathology, 49*, 317–343.

Sarowar, S., Kim, Y., Kim, E., Kim, K., Hwang, B., Islam, R., et al. (2005). Overexpression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses. *Plant Cell Reports, 24*, 216–224.

Subba Rao, M. V., & Muralikrishna, G. (2002). Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (ragi, *Eleusine coracana* Indaf-15). *Journal of Agricultural and Food Chemistry, 50*, 889–892.

Supek, F., Bosnjak, M., Skunca, N., & Smuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One, 6*.

Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., et al. (2004). MAPMAN: A user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal, 37*, 914–939.

Thomas, P. D., Campbell, M. J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., et al. (2003). PANTHER: A library of protein families and subfamilies indexed by function. *Genome Research, 13*, 2129–2141.

Thomma, B. P. H. J., Eggermont, K., Penninckx, I. A. M. A., Mauch-Mani, B., Vogelsang, R., Cammue, B. P. A., et al. (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 15107–15111.

van Loon, L. C., Rep, M., & Pieterse, C. M. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology, 44*, 135–162.

Xu, G., Ye, X., Chen, J., & Liu, D. (2007). Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. *Journal of Agricultural and Food Chemistry, 55*, 330–335.