FINAL PROJECT REPORT
WTFRC Project Number: AH-04-420

Project Title: Target-specific control of fungal pathogens of tree fruit by natural compounds

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	14,192	14,618	15,056
Benefits			
Wages			
Benefits			
Equipment	5,000	3,000	2,800
Supplies	2,700	4,000	4,000
Travel	800	800	
Miscellaneous			
Total	22,692	22,418	21,856

[1]Salary for one half-time GS5 Biological Lab. Tech. with 3% projected salary increase for FY05 and 06. [2]2004-Upgrade DNA sequencer; 2005- Plant growth chamber; 2006- PCR thermo-cycler [3]2004- Yeast & fungal strains, kits (nucleic acids work), oligos, plasmids; 2005 & 2006- kits (nucleic acids & protein works), oligos, plasmids, fungal growth media, chemicals. [4]2004 & 2005- Washington State, field trip to orchards to isolate pathogens. [5]Technical support, equipment and supplies are being requested in this proposal for added ability to research on functional genomics and control of phytopathogenic fungi of orchard crops.

Objectives:

Identify new natural compounds effective as antifungals. In apple orchards, controlling a phytopathogenic disease is problematic as chemical controls are currently very limited for several fungal diseases. In the past three years of study, we identified a set of natural compounds with great promise for controlling fungal pathogens. We proved antioxidative stress response/defense systems are essential for fungal tolerance to the natural compounds identified and are potentially useful molecular targets for control of fungal pathogens such as *Penicillium*. We also focused on developing/determining an effective method for delivery of newly discovered natural compounds, leading to a target-specific strategy for an easy, safe and economic approach to pathogen control.

Significant Findings (Last 3 years):

- Molecular targets of identified natural compounds were determined using yeast *Saccharomyces cerevisiae* as a model fungal system.
- Selected phenolic agents were used in target-gene based bioassays in combination with conventional fungicides:

  Mitochondrial superoxide dismutase (Mn-SOD) as a target:

  (1) Targeting Mn-SOD with phenolics such as **vanillylacetone** resulted in a 100 to 1000 fold greater sensitivity to **strobilurin** (inhibitors of complex III of the mitochondrial respiratory chain) or **carboxin** (inhibitors of complex II of the mitochondrial respiratory chain) fungicides. This synergism is significantly greater with strobilurin than with carboxin, suggesting that complex III is a better target than complex II for fungal control, using phenolics.

  (2) Enhancement of antifungal activity of strobilurins was tested using both **berberine** (an alkaloid) and phenolic compounds. With berberine, the most effective phenolic was **veratraldehyde**. The *sod2*Δ mutant (Mn-SOD mutant) of *S. cerevisiae* was highly sensitive to berberine and veratraldehyde. Functional complementation analysis verified these compounds target Mn-SOD.

  (3) Activity of **strobilurin** (25 to 50 μM) was significantly elevated on *Aspergillus fumigatus* and *A. nidulans* by co-application with berberine (2 mM) and/or by veratraldehyde (2-4 mM) on *Penicillium expansum* and most aspergilli.

  Antioxidative signal transduction (MAPK) or vacuolar transport system as targets:

  (1) These compounds also prevented *A. fumigatus* mitogen-activated protein kinase (MAPK) mutants from escaping toxicity of **fludioxonil**, a phenylpyrrole fungicide potentiated by the MAPK pathway that regulates osmotic/oxidative stress-responses in fungi.

  (2) Activity of fludioxonil is elevated by co-application of the aspirin/salicylic acid metabolite, **2,5-dihydroxybenzoic acid** (2,5-DHBA). 2,5-DHBA disrupts cellular GSH (reduced glutathione)/GSSG (oxidized glutathione) homeostasis, further stressing the oxidative stress-response system, enhancing fludioxonil activity. The 2,5-DHBA treatment also prevents tolerance of MAPK mutants resistant to fludioxonil.

  (3) Positive interaction between phenolics and **concanamycin A**, an inhibitor of V-ATPase, or berberine was observed where combined application of test phenolics with either of these compounds greatly enhanced the inhibition of fungal growth.
- These results show certain natural compounds are effective antifungals or synergists to conventional fungicides and can be used for improving control of food-contaminating pathogens. Use of such compounds for fungal control reduces environmental and health risks associated with commercial fungicides, and lowers cost for control and the probability for development of resistance to these fungicides.

Methods:

***In vitro* susceptibility bioassays.** Phenolics (*i.e.,* cinnamic, *o*-coumaric, *m*-coumaric, *p*-coumaric, caffeic, ferulic, benzoic, vanillic, 4-hydroxybenzoic, 3,4,5-trimethoxybenzoic, 3-chlorobenzoic and salicylic acids, benzaldehyde, veratraldehyde, vanillin, vanillylacetone, thymol), carboxin, strobilurins and berberine hemisulfate were examined in fungi. For yeast assays, ~10^6 cells were cultured in YPD (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) and serially diluted from 10-fold to 10^5-fold in SG (0.67% Yeast nitrogen base w/o amino acids, 2% glucose with appropriate supplements: 0.02 mg/ml uracil, 0.03 mg/ml amino acids). Cells from each serial dilution were spotted adjacently on SG agar medium incorporated with each phenolic to be tested. Cells were grown at 30 ^oC for 7 days. Numerical scoring is: 6= colonies were visible in all dilutions, 0= no colonies were visible in any dilution, 1= only the undiluted colony was visible, 2= the undiluted and 10-fold diluted colonies were visible, *etc.* Sensitivity of *S. cerevisiae* was also tested with the modifications of the guidelines of NCCLS document M27-A (National Committee for Clinical Laboratory Standards, 1997). An inoculum size of ~ 5 X 10^3 colony forming units (CFU)/ml yeast cells were incubated in flat-bottomed microtiter plates in SG liquid medium (200 µl/well; 30 ^oC) containing phenolics, and the spectrophotometric reading (OD at 600 nm) was performed after 48 h of incubation. All treatments were performed in triplicate. For fungal assays ~200 spores were diluted in Phosphate-Buffered Saline (PBS) and spotted in the center of Potato Dextrose Agar (PDA) plates containing phenolic reagents and/or inhibitors of mitochondrial respiration and cell growth was monitored after 7 days at 28 ^oC (37 ^oC for *A. fumigatus*). Colony growth was measured based on percent radial growth compared to control colonies.

***S. cerevisiae sod2*Δ complementation bioassay.** We examined if complementation of the yeast *sod2*Δ mutant lacking Mn-SOD gene with the orthologous fungal gene (*i.e*., *sodA* from *A. flavus*; encodes Mn-SOD; GenBank accession# AY585205) reversed any effects observed in the *sod2*Δ mutant to berberine hemisulfate and phenolics. This complementation bioassay was done as described previously to examine effects of compounds on Mn-SOD (Kim *et al.* 2005). *S. cerevisiae sod2*Δ with pYES2 empty vector (*sod2*Δ + pYES2; negative control; Invitrogen), wild type with pYES2 empty vector (WT + pYES2; positive control), wild type with pYES2 vector containing PCR-amplified *sodA*, the *A. flavus* Mn-SOD gene (WT + *sodA*; Mn-SOD overexpression), and *sod2*Δ with pYES2 vector containing PCR-amplified *sodA* (*sod2*Δ + *sodA*; functional complementation) were cultured in raffinose medium (0.67% Yeast nitrogen base without amino acids, 110 µM raffinose, 200 µM amino acids) at 30 ^oC. Yeast cells were serially diluted as described above with raffinose liquid medium and spotted adjacently on SGAL (0.67% Yeast nitrogen base w/o amino acids, 110 µM galactose, 200 µM amino acids). Functional expression of *sodA* was achieved under the yeast *GAL1* promoter (30 ^oC, 10 days). Functionality of *sodA* was assessed based on yeast cell dilution having visible growth in the presence of berberine hemisulfate and phenolics. If the dilution showing yeast growth was similar to the positive control or better than the negative control, *sodA* was considered to have been functionally complemented *sod2*Δ. Reduced sensitivity after complementation would signify test compounds targeted the yeast oxidative stress response system, *i.e*., Mn-SOD.

Enhanced growth inhibition of fungi by co-application of test compounds: synergistic inhibition. Phenolic compounds were added to the medium with strobilurin, carboxin, concanamycin A (vacuolar ATPase inhibitor) or berberine hemisulfate. The cell growth was monitored for 5 to 7 days at 30 ^oC or 28 ^oC for yeast or fungal pathogens, respectively. The types of medium, culture condition and measurement of the cell growth were as described above. To test the positive interaction, *i.e*., synergistic inhibition, with concanamycin A, *o*-coumaric acid was chosen and combined in the medium for target fungi (*Penicillium,* aspergilli, and yeast). Positive interaction between berberine hemisulfate (0.5 mM) and phenolics (vanillylacetone 10 mM, veratraldehyde 5 mM, vanillic acid 3 mM, vanillin 1 mM, cinnamic acid 0.1 mM, *m*-coumaric acid 5mM) were tested in the yeast as described above. For other fungi, *i.e*., *Penicillium* and aspergilli, berberine hemisulfate (0.5 or 1 mM) and vanillylacetone (5 or 10 mM) were given together in the PDA medium, and the cell growth was monitored for 5 to 7 days.

Results and discussion:

Identification of molecular targets of phenolic agents in the model yeast *S. cerevisiae.* As an approach to identify vulnerable gene targets in pathogenic fungi, we first tested the levels of sensitivity in our model fungal yeast system. We examined forty-six *S. cerevisiae* deletion mutants defective in the antioxidative stress response system against seventeen phenolic agents (Kim *et al.* 2005). We chose minimum effective concentration (MEC) for each compound where the growth of the wild type yeast was almost not affected but reduced colony size. Molecular target(s) most crucial for cellular tolerance/detoxification against the antifungal phenolics could be identified under this condition. Four classes of mutants showing hypersensitivity to the test compounds were identified, as follows: i) regulators of pH responsive transcription (*rim101*Δ) or glutathione transferase transporter (*ure2*Δ), ii) V-ATPase system (*tfp1/vma1*Δ, *vph2*Δ), iii) MAPK kinase (*hog4*Δ; MAPKK), and iv) antioxidative enzymes for glutathione biosynthesis (*gsh1*Δ, *gsh2*Δ)/superoxide dismutation (*sod1*Δ, *sod2*Δ). Genes identified are suggested to be important molecular targets for control of phytopathogenic fungi. The levels of antifungal activity of phenolics were not correlated to their acidic nature, but were due to certain structural characteristic(s).

Identification of an effective antifungal target on the mitochondrial respiratory chain. Phenolics such as vanillylacetone affect the normal function of mitochondrial respiration of yeast (Kim *et al.* 2006). To target mitochondrial respiration and the antioxidative stress response system more efficiently for fungal control, we applied vanillylacetone to synergize the effects of the fungicides carboxin or strobilurin. Application of vanillylacetone enhanced the level of growth inhibition by these fungicides (**Table 1**). There was greater synergism in activity of strobilurin than that of carboxin by vanillylacetone, suggesting complex III of the respiratory chain is a more efficient target for fungal control. Thus, using vanillylacetone as a synergist may significantly reduce potential for development of resistance to these types of fungicides that inhibit mitochondrial respiration; a frequent problem with conventional fungicides.

Table 1. Effects of different concentrations of fungicides (μM) carboxin and strobilurin combined with vanillylacetone (mM) on growth of fungi *Penicillium expansum* (*Pe*), *Aspergillus niger* (*An*) and *A. flavus* (*Af*)[a].

Vanillylacetone (mM)	0	10	15	20
Fungal species	*Pe An Af*	*Pe An Af*	*Pe An Af*	*Pe An Af*
Fungicide (μM)				
None 0	100,100,100	64, 60, 91	40, 19, 58	24, 0, 29
Carboxin 50	75, 100, 98	29, 32, 64	~0, 17, 29	0, 0, 0
100	71, 100, 96	19, 24, 47	0, 0, 17	0, 0, 0
Strobilurin 50	71, 100,100	0, 0, 18	0, 0, 0	0, 0, 0
100	69, 100, 98	0, 0, 0	0, 0, 0	0, 0, 0

[a] Growth of fungi is represented as a percentage of radial growth of the fungal mat of treated compared to control (no inhibitors of mitochondrial respiratory chain). Values are means of three replicates, standard deviations of all measurements were <2%. ~0 means barely germinated.

Identification of berberine analog as a natural synergist for targeting mitochondrial superoxide dismutase (Mn-SOD). Berberine targets the activity of Mn-SOD. Berberine also acts as a synergist for fungal control with phenolics. The antifungal interaction between berberine (0.5, 1 mM) and veratraldehyde (2.5, 5 mM) on several different aspergilli and *P. expansum* showed that germination was completely inhibited in *A. fumigatus*, *A. parasiticus*, *A. oryzae*, *A. niger* and *A. nidulans* at 0.5 mM berberine (1 mM for *A. niger*) and 5 mM veratraldehyde (**Table 2**). However, the growth of *A. flavus*, *A. ochraceous* and *P. expansum* was reduced but not completely inhibited at the highest doses tested of both compounds (**Table 2**).

Table 2. Relative growth of *P. expansum* and aspergilli treated with varying amounts of veratraldehyde or berberine hemisulfate, individually or in combination*

Veratraldehyde (mmol l^{-1})	0			2.5			5		
Berberine (mmol l^{-1})	0	0.5	1.0	0	0.5	1.0	0	0.5	1.0
A. fumigatus AF293	100	60±17	56	100	0	0	94	0	0
A. flavus NRRL 3557	100	98	94	98	94	83	90	73	0
A. parasiticus NRRL 5862	100	96	92	90	76	60	72	0	67
A. ochraceous NRRL 5175	100	85	81	88	73	63	69	50	0
A. oryzae FGSC A815	100	89	83	87	62	51	70	0	48
A. niger NRRL326	100	80	78	106	84	78	80±27	64±13	0
A. nidulans FGSC A4	100	76	64	84	0	0	0	0	0
P. expansum NRRL 974	100	85	81	100	81	74	89	59	52

* Fungal growth is presented as a percentage of radial growth compared to control colonies grown on PDA plates receiving only DMSO. Values are means of three replicates. Standard deviations of all measurements are <5%, except where noted.

The *sodA*/*sod2*Δ complementation assay (See Kim *et al.* 2005 for the method) showed that Mn-SOD plays a role in tolerance to berberine and phenolics. The *sod2*Δ mutant overcame berberine/phenolic-mediated growth inhibition by functional expression of *Aspergillus sodA*. Co-application of berberine and veratraledehyde or vanillylacetone greatly reduced yeast survivability in all strains, including the wild type, compared to individual treatment of these compounds (**Figure 1**). The *sod2*Δ complemented with the functional *sodA* gene (*sod2*Δ-*sodA*) had the same level of growth as the wild type when exposed to berberine and the phenolics. The *sod2*Δ with the empty vector, however, was 10 to 100 times more sensitive to these compounds. The *sod2*Δ-*sodA* complementation also resulted in greater tolerance to individual treatments of veratraldehyde or vanillylacetone. Over-expression of Mn-SOD (wild type + *sodA*) had no effect on hypertolerance. This result further demonstrates Mn-SOD plays an important role for fungal tolerance to our test compounds.

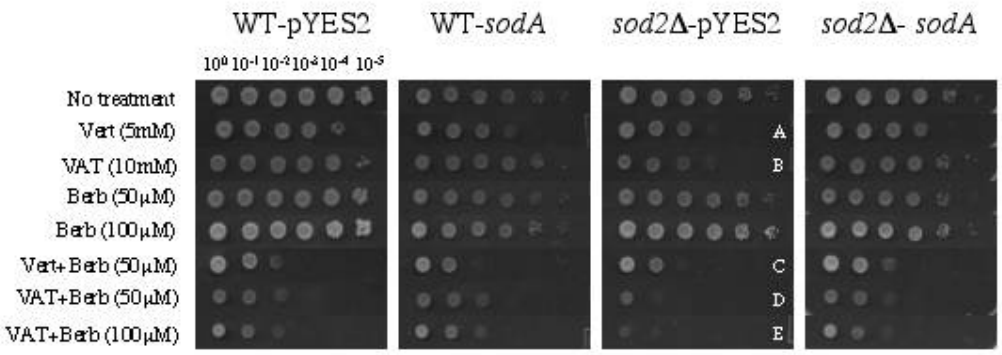

Figure 1 Functional complementation assay of the Mn-SOD gene from *Aspergillus flavus* (*sodA*), using the vector pYES2, in yeast wild type (WT) or *sod2*Δ, lacking the orthologous gene. Assay shows *sod2*Δ-pYES2 (no Mn-SOD + empty vector) is 10 to 100 times more sensitive to 5 mM veratraldehyde (Vert) (A) and 10 mM vanillylacetone (VAT) (B) and to combined treatments with berberine (Berb) (C, D and E) than complemented *sod2*Δ-*sodA* and wild type (WT) strains.

2,5-DHBA inhibits fungal growth by disrupting cellular glutathione homeostasis. 2,5-Dihydroxybenzoic acid (2,5-DHBA) is a cellular metabolite of salicylic acid (2-hydroxybenzoic acid), in turn a metabolite of aspirin (acetylsalicylic acid) (Forth *et al*., 1987). Lower doses of 2,5-DHBA relieved oxidative stress in *S. cerevisiae* in accordance with prior evidence showing that 2,5-DHBA had antioxidant activity (Ashidate *et al*., 2005; See **Figure 2**). We tested the antifungal activity of 2,5-DHBA against 46 additional yeast deletion mutants, using the dilution bioassay. These mutants were previously identified as lacking various genes in the oxidative stress response pathway (see Materials and methods; Kim *et al*., 2005). Of these 46 strains, *ure2*Δ (putative glutathione transferase mutant), *vph2*Δ (vacuolar H(+)-ATPase assembly mutant), *ste20*Δ (protein ser/thr kinase mutant), *glr1*Δ (glutathione reductase mutant), *gsh1*Δ (γ-glutamylcysteine synthetase mutant), *gsh*2Δ (glutathione synthetase mutant), *sod1*Δ (Cu/Zn superoxide dismutase mutant), *sod2*Δ (Mn superoxide dismutase mutant), *hog1*Δ (MAPK mutant) and *hog4*Δ (MAPK kinase mutant) were 10 to 1,000 times more sensitive than the wild type, when exposed to 6 to 18 mM 2,5-DHBA. Based on this observation using *S. cerevisiae* as a model system, we concluded that these 10 genes, or their orthologs, played relatively more significant roles than other fungal genes, in responding to or tolerating toxic levels of 2,5-DHBA.

Supplementation of GSH at 0.1 mM resulted in almost complete recovery of the *glr1*Δ strain to 2,5-DHBA-induced toxicity, whereas supplementation of GSSG had no effect (**Figure 2**). This response in recovery of the *glr1*Δ strain to GSH and not GSSG suggested that 2,5-DHBA may play a role in disrupting regulation of reduced *vs.* oxidized levels of glutathione in the cell.

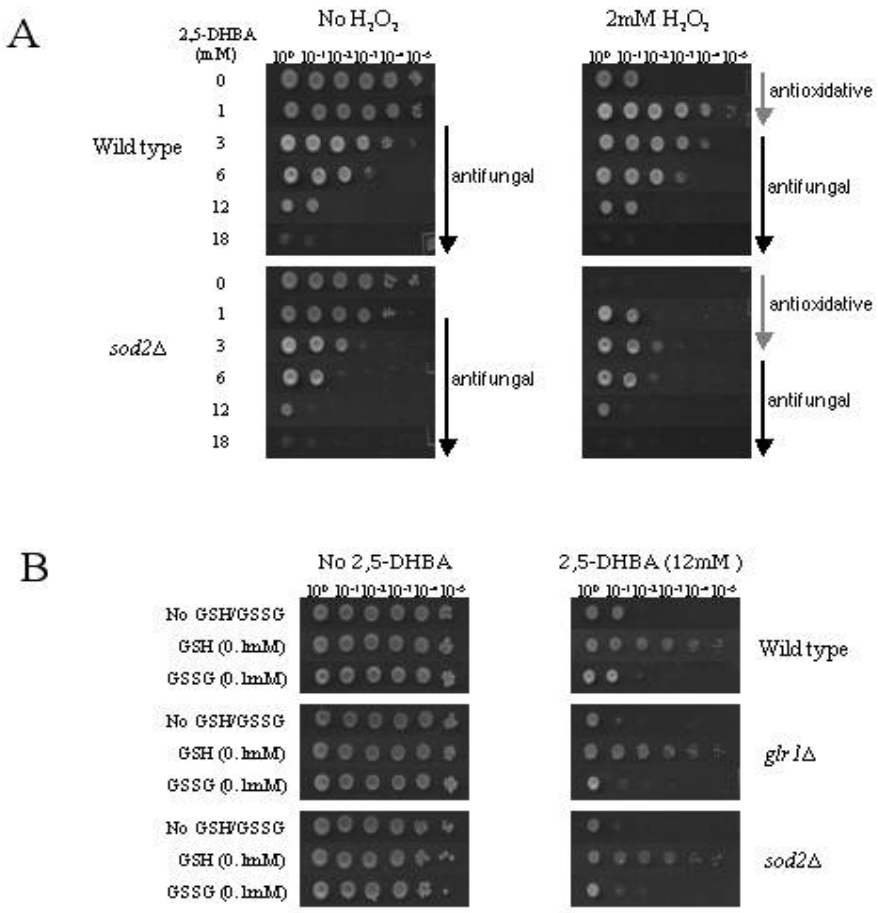

Figure 2. (**A**) Antioxidant/antifungal activities of 2,5-DHBA using a yeast bioassay based on 10-fold serial dilutions of yeast cultures placed sequentially on the growth medium. For testing antioxidant activity of the compounds, different concentrations of hydrogen peroxide (H_2O_2; 1, 2, 3 mM) were applied to the culture medium and the antioxidant/antifungal effects of 2,5-DHBA were monitored as described in the text. The representative bioassays shown here are of the 2 mM H_2O_2 treatment. (**B**) Yeast bioassay showing treatment with GSH (0.1, 0.5 mM) induced recovery of cell growth (wild type, *glr1*Δ, *sod2*Δ) from 2,5-DHBA toxicity, whereas GSSG (0.1, 0.5 mM) did not. This shows 2,5-DHBA prevents reduction of GSSG to GSH. The representative bioassays shown here are of the 0.1 mM GSH/GSSG treatment.

2,5-DHBA enhances fludioxonil activity in wild type and MAPK mutants of fungi. Certain fungi having MAPK mutations are tolerant to phenylpyrrole fungicides (Kojima *et al*., 2004). Both *sakA*Δ/*mpkC*Δ MAPK mutants of *A. fumigatus* escaped fludioxonil toxicity (**Figure 3A**). However, we found co-applying 2,5-DHBA with fludioxonil prevented these mutants from developing tolerance to this fungicide (**Figure 3A**). Presumably, this tolerance is prevented because 2,5-DHBA targets genes downstream in these MAPK pathways targeted by fludioxonil and destabilizes GSH/GSSG homeostasis (**Figure 3B**). Considering MAPK signaling pathway is highly conserved in different organisms, we are currently focusing on this pathway in *Penicillium* or other apple pathogens as a target for effective fungal control.

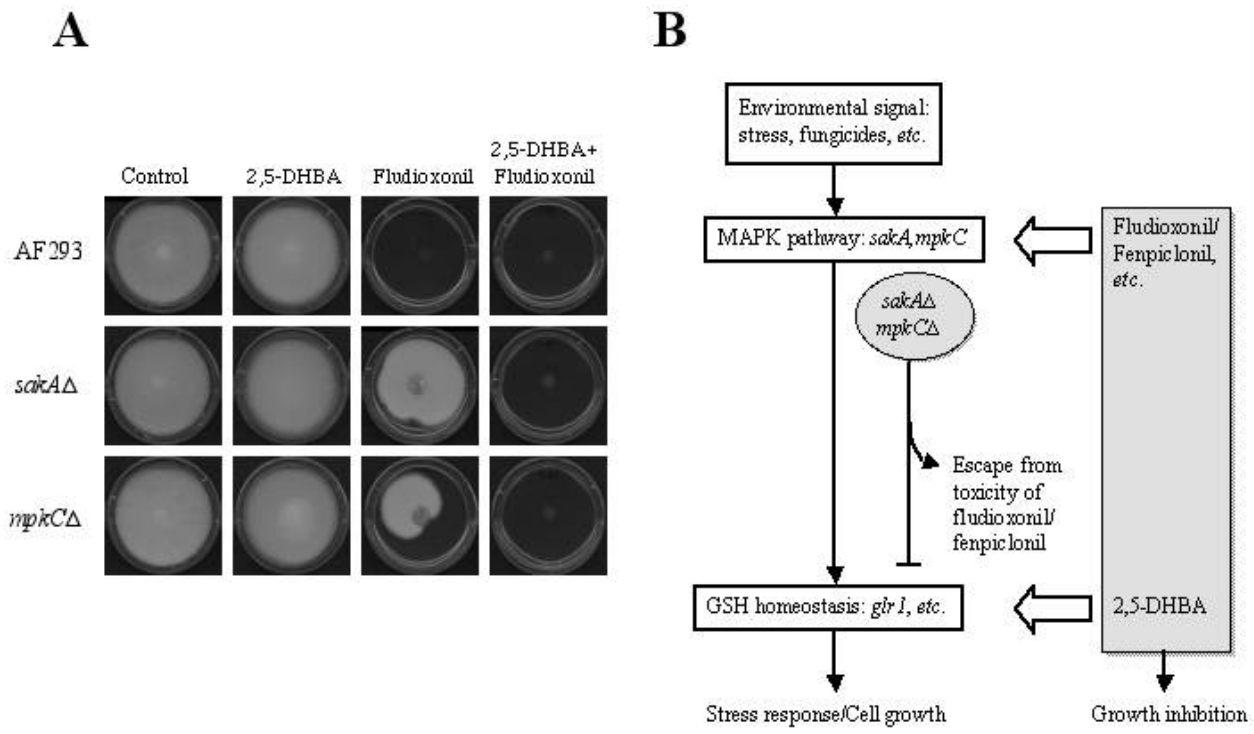

Figure 3. Inhibiting tolerance (escape) of *sakA*Δ and *mpkC*Δ MAPK mutants of *A. fumigatus* to phenylpyrrole fungicides by co-application of 2,5-DHBA. (**A**) Representative bioassays of wild type (AF293) and MAPK mutants with no treatment (Control), 2,5-DHBA (12 mM), fludioxonil (50 μM) and fludioxonil (50 μM) + 2,5-DHBA (12 mM). Note that co-treatment of 2,5-DHBA prevents *sakA*Δ and *mpkC*Δ MAPK mutants from escaping fludioxonil toxicity. (**B**) Scheme showing where phenylpyrrole fungicides (*e.g.*, fludioxonil) target MAPK signaling pathway genes. MAPK mutants escape toxicity by missing the signal stimulated by phenylpyrrole fungicides and, thus, avoiding the induced osmotic/oxidative stress response. Application of 2,5-DHBA disrupts cellular GSH homeostasis, which enhances the toxicity in the wild type cells or helps prevent escape of MAPK mutants from antifungal effects.

We also compared the activity of structural derivatives of 2,5-DHBA on *Penicillium expansum* and more species of aspergilli. Acetylsalicylic acid had the highest antifungal activity in all fungi tested. 2,3-DHBA and 2,4-DHBA/2,5-DHBA showed decreased toxicity, respectively (**Table 3**). 3,4-DHBA had little antifungal activity, even at the highest concentration (21 mM). In a separate assay, benzoic acid (no –OH group on the aromatic ring) had the highest antifungal activity, followed by 2-hydroxybenzoic (salicylic) acid and acetylsalicylic acid. These responses, based on chemical structure, indicate additional hydroxyl groups, and further away from the ortho position in the aromatic ring, gradually decrease antifungal activity.

Table 3. Structure-activity relationship of effect of dihydroxybenzoic acid (DHBA) derivatives on growth of *P. expansum*, *A. fumigatus* and *A. flavus*[1].

Conc. (mM)	Control	Acetyl-salicylic acid	2,3-DHBA	2,4-DHBA	2,5-DHBA	3,4-DHBA
A. fumigatus						
0	100	-	-	-	-	-
9	-	0	22	72	76	92
12	-	0	0	44	58	92
15	-	0	0	46	44	92
18	-	0	0	32	28	92
21	-	0	0	0	12	90
A. flavus						
0	100	-	-	-	-	-
9	-	60	88	92	80	96
12	-	0	84	80	74	96
15	-	0	0	72	60	96
18	-	0	0	42[2]	42	98
21	-	0	0	0	0	98
P. expansum						
0	100	-	-	-	-	-
9	-	52	74	77	77	90
12	-	0	58	68	58	87
15	-	0	0	52	48	81
18	-	0	0	23	32	81
21	-	0	0	0	23	77

[1] Responses are percentage of radial growth compared to control colonies grown on PDA plates receiving only DMSO. Values are means of three replicates. Standard deviations of all measurements are <3% except where noted. For fungal assays, ~200 spores were diluted in phosphate-buffered saline (PBS) and spotted onto the center of potato dextrose agar (PDA) plates containing test compounds and incubated at 37 ^{o}C and 28 ^{o}C for *A. fumigatus* and *A. flavus*/*P. expansum*, respectively, for 5 days. *A. flavus* NRRL3357 and *P. expansum* NRRL974 were obtained from National Center for Agricultural Utilization and Research, USDA, Peoria, IL (http://nrrl.ncaur.usda.gov/index.html) and A. fumigatus strains were kindly provided by Dr. Greg May (University of Texas M.D. Anderson Cancer Center).

[2]Standard deviation: 4%

Summary. During last three years we identified a potentially effective approach to fungal control using newly discovered natural compounds that have a target-specific basis of activity. Antioxidative stress response systems of fungi can be an efficient molecular target of phenolics for pathogen control. We proved positive interaction between phenolics and conventional fungicides or berberine significantly augment the fungicidal effects of commercial fungicides. Certain phenolics disrupt cellular redox homeostasis by targeting the fungal antioxidative stress systems. We also showed how 2,5-DHBA greatly improved effectiveness of fludioxonil, a phenylpyrrole fungicide. Our results indicate this improvement is from the ability of 2,5-DHBA to disrupt glutathione homeostasis, resulting in cellular GSH/GSSG imbalances. Such supplementation, using safe, natural compounds to augment effectiveness of commercial fungicides or antifungal drugs, lowers dosages of commercial fungicides required for effective control. Consequently, this lower dosage reduces environmental impact and risks to human health by lowering exposure to fungicides. Additionally, there is decreased potential for development of fungal resistance. We conclude natural compounds such as phenolic agents that do not have any significant medical or environmental shortcomings could be useful in control programs involving conventional antifungal agents.

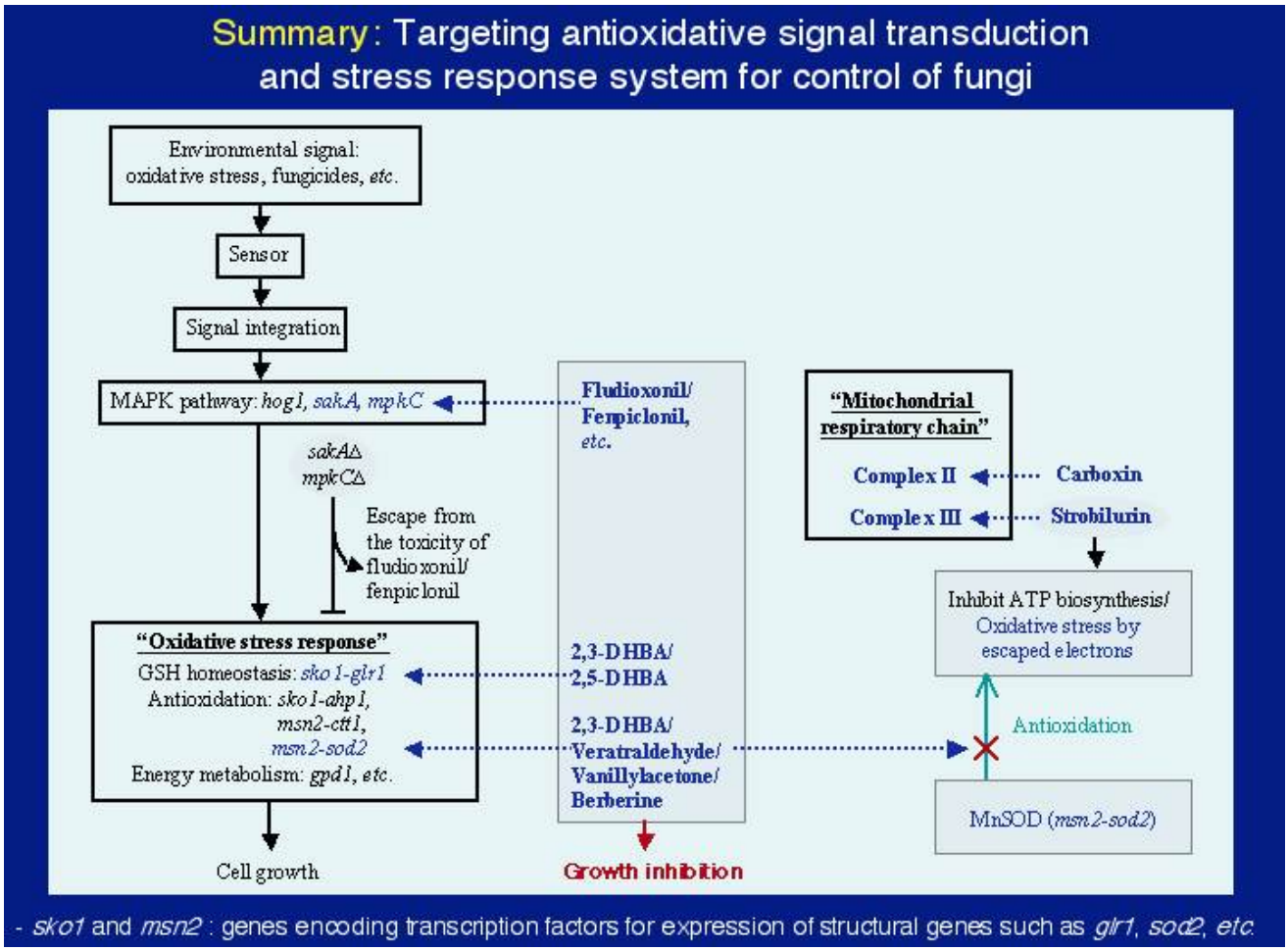

Figure (Summary). Elucidation of targeting antioxidative signal transduction and stress response system for control of fungi. DHBA, dihydroxybenzoic acid. See text for the description of other genes.

References:

Ashidate, K., Kawamura, M., Mimura, D., Tohda, H., Miyazaki, S., Teramoto, T., Yamamoto, Y. and Hirata, Y. 2005. Gentisic acid, an aspirin metabolite, inhibits oxidation of low-density lipoprotein and the formation of cholesterol ester hydroperoxides in human plasma. *Eur J Pharmacol* **513:** 173–179.

Bennis, S., Chami, F., Chami, N., Bouchikhi, T. and Remmal, A. 2004. Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. *Lett. Appl. Microbiol.* **38:** 454-458.

Forth, W., Henschler, D. and Rummel, W. 1987. Allgemeine und spezielle pharmakologie und toxikologie. Wien, Zurich: Wissenschaftsverlag, Mannheim.

Kim, J.H., Campbell, B.C., Yu, J., Mahoney, N., Chan, K., Molyneux, R.J., Bhatnagar, D. and Cleveland, T.E. 2005. Examination of fungal stress response genes using *Saccharomyces cervisiae* as a model system: targeting genes affecting aflatoxin biosynthesis by *Aspergillus flavus* Link. *Appl. Microbiol. Biotechnol.* **67:** 807-815.

Kim, J.H., Mahoney, Chan, K.L., Molyneux, R.J. and Campbell, B.C. 2006. Controlling food-contaminating fungi by targeting their antioxidative stress-response system with natural phenolic compounds. *Appl. Microbiol. Biotechnol.* **70:** 735-739.

Kojima, K., Takano, Y., Yoshimi, A., Tanaka, C., Kikuchi, T. and Okuno, T. 2004. Fungicide activity through activation of a fungal signalling pathway. *Mol Microbiol* **53:** 1785–1796.