**Mechanism of action of dicarboximide and phenylpyrrole on the stress-response signal transduction pathway**

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The mechanism of action of dicarboximides and phenylpyrroles has been studied in *Neurospora crassa*. Both fungicides were found to interfere with the signal transduction pathway composed of the following components: osmotic-sensitive (OS)-1 histidine kinase, histidine phosphotransfer protein (HPT)-1, response regulator protein (RRG)-1, OS-4 mitogen-activated protein kinase (MAPK) kinase kinase, OS-5 MAPK kinase, and OS-2 MAPK. All the components, except HPT-1, were essential for the sensitivity to both fungicides and adaptation to osmotic stress. In contrast, the hpt-1 deletion mutation was lethal unless OS-2 was inactivated. Fludioxonil, osmotic stress, and heat shock induced OS-2 activation by phosphorylation. OS-2 regulated various genes, such as gcy-1, which encodes glycerol dehydrogenase; cat-1, which encodes conidial catalase; and the clock-controlled gene (ccg)-1. This implies that the signaling pathway plays an important role not only in the stress response but also in asexual differentiation and circadian output. We found 3 types of dicarboximide-resistant mutations in the *BcOS1* gene of *Botrytis cinerea*, of which the I365S mutation was dominant in the fields. A hybridization probe assay was developed to detect these mutations in a single polymerase chain reaction that may be suitable for monitoring the development of resistance to various fungicides. © Pesticide Science Society of Japan

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glycerol accumulation in the wild-type strain, but not in the \textit{os} mutants.

Glycerol accumulation in response to hyperosmolarity is an important mechanism for maintaining turgor pressure in the cells. Osmoregulation mediated by the histidine kinase \textit{Slr1p} has been well characterized as a high-osmolarity glycerol (HOG) pathway in \textit{Saccharomyces cerevisiae}. Although budding yeast cells are resistant to iprodione and fludioxonil, a similar signaling pathway in fungi is thought to be the predominant target of fungicides. Studies conducted by our group and others revealed that \textit{os} genes in \textit{N. crassa} encode components for a pathway similar to the HOG pathway in \textit{S. cerevisiae}. The two-component signal transduction pathway in \textit{Neurospora} comprises the His-Asp phosphorelay system and the mitogen-activated protein kinase (MAPK) cascade. The phosphorelay system includes \textit{OS-1}, a sensor histidine kinase; \textit{HPT-1} a His-containing phosphotransfer (HPt) protein; and \textit{RRG-1}, a receiver response regulator. The MAPK cascade includes \textit{OS-4} MAPKK kinase, \textit{OS-5} MAPK kinase, and \textit{OS-2} MAPK. Among them, we identified and characterized \textit{hpt-1}, \textit{os-4}, and \textit{os-5} genes. The \textit{os-4} and \textit{os-5} gene mutants had two contrasting phenotypes: hypersensitivity to osmotic stress and resistance to iprodione and fludioxonil; this indicated that proper functioning of this signal transduction pathway is essential for sensitivity to these antifungal compounds.

\textit{N. crassa} has 11 putative histidine kinase genes, including those similar to the yeast \textit{SLN1} gene. Among all these genes, mutation in only \textit{os-1} confers the properties of fungicide resistance and osmotic sensitivity. \textit{S. cerevisiae} has only one histidine kinase \textit{SLN1} gene, and disruption of this kinase is lethal because the Hog1 MAPK pathway is constitutively activated. In contrast, the \textit{os-1} gene is not essential for the growth of \textit{N. crassa} under normal hypotonic conditions. \textit{OS-1} has 6 unique 90-amino acid repeats in the sensor region. The \textit{os-1} allele mutants, with mutations in these amino acid repeats, exhibited the pleiotropic phenotypes of fungicide resistance and osmosensitivity. The \textit{hpt-1} gene, which encodes only HPt, was successfully disrupted in the \textit{os-2} MAPK mutant, but never in the wild-type strain. Genetic analysis revealed that \textit{hpt-1} is an essential gene under normal growth conditions and its lethality is suppressed by \textit{os-2} or \textit{os-5} mutation.

Fludioxonil and iprodione treatments induced the phosphorylation of \textit{OS-2} MAPK. In addition, \textit{OS-2} was activated in response to multiple stress conditions, including osmotic stress, heat shock, and oxidative stress; however, \textit{OS-4} MAPKK kinase and \textit{OS-5} MAPK kinase were essential for \textit{OS-2} phosphorylation under such multiple stress conditions. In contrast, \textit{OS-1} histidine kinase was essential for \textit{OS-2} phosphorylation in the presence of fungicides and at low levels of osmotic stress, but this enzyme was not essential under high osmotic and other stresses. The results suggest that these fungicides may exert their antifungal property by overactivating the MAPK cascade downstream of \textit{OS-1} histidine kinase.

\textit{OS-2} MAPK was found to regulate various types of genes that function in the osmotic stress response and cell differentiation. Fludioxonil and osmotic stress induced the synthesis of glycerol by upregulating the expression of \textit{gcy-1}, which encodes a putative glycerol dehydrogenase; this upregulation results in increased enzyme activity of this dehydrogenase. \textit{N. crassa}, like other filamentous fungi, has specific fungal catalases, CAT-1 and CAT-3, predominant in the conidia and mycelia, respectively. Fludioxonil and osmotic stress also induce the expression of \textit{cat-1} and its corresponding enzyme activity in the mycelia in an \textit{OS-2}-dependent manner. In addition, several clock-controlled genes, such as \textit{ccg-1}, \textit{ccg-13}, and \textit{bli-3}, are upregulated by \textit{OS-2} activation. These findings imply that the stress-response MAPK cascade plays an important role not only in the osmotic stress response but also in cell differentiation in filamentous fungi.

### Identification of the mutations causing resistance to dicarboximides in \textit{B. cinerea}

In contrast to the \textit{os} mutants of \textit{N. crassa}, dicarboximide-resistant field isolates of \textit{B. cinerea} did not show osmosensitiv-
Conclusion

The mechanisms of action of dicarboximides and resistance to dicarboximides remained unclear even after almost 30 years of research. Our studies indicated that dicarboximides and phenylpyrroles have a unique mode of action; i.e., these fungicides target the stress-response signal transduction pathway. Recently, this pathway has been studied in various filamentous fungi, and these fungicides are being used as a molecular tool to activate this pathway. Further, we identified the histidine kinase $BcOS1$ mutations that confer dicarboximide resistance in field isolates of $B. \text{cinerea}$. Dicarboximide-resistant strains with the I365S mutation in the $BcOS1$ gene are distributed worldwide. Genotyping of field isolates in Japan suggested that the frequency of dicarboximide-sensitive isolates is increasing because of the decreasing use of dicarboximides as fungicides; this is probably because of the introduction of new fungicides, such as anilinopyrimidines, hydroxyanilides, and QoI fungicides.