Title Superoxide anion and hydrogen peroxide in the yeast antagonist–fruit interaction: A new role for reactive oxygen species in postharvest biocontrol?

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Abstract

The importance of reactive oxygen species (ROS) in plant defense responses against certain pathogens is well documented. There is some evidence that microbial biocontrol agents also induce a transient production of ROS in a host plant which triggers local and systemic defense responses to pathogens. The ability of biocontrol agents used to control postharvest diseases to induce defense-related oxidative responses in fruits, however, has not been explored. Here we show that the yeast antagonists, *Metschnikowia fructicola* (strain 277) and *Candida oleophila* (strain 182) generate greater levels of super oxide anion (O_2^-) on intact fruit surfaces (poor in nutrients) then those applied on a nutrient-poor agar medium. Even though yeast antagonists show a high level of O_2^- on nutrient-rich media, when applied on fruits around wounds (areas abundant in nutrients) accumulation of O_2^-, as detected by nitro blue tetrazolium staining, occurred much more rapidly on the latter. Using laser scanning confocal microscopy we observed that the application of *M. fructicola* and *C. oleophila* into citrus and apple fruit wounds correlated with an increase in H_2O_2 accumulation in host tissue. In citrus fruit, the level of H_2O_2 around inoculated wounds increased by 4-fold compared to controls (wounds inoculated with water) as early as 18 h after inoculation. Yeast continued to stimulate H_2O_2 production in citrus fruit up to 66 h after inoculation and H_2O_2 levels were still 3-fold above the control. Living yeast cells were detected in fruit wounds at this time point indicating the ability of *M. fructicola* to tolerate host ROS, which has been reported to be an intrinsic characteristic of efficient yeast antagonists. Similar increase in H_2O_2 accumulation around yeast-inoculated wounds was observed in apple fruit exocarp. The present data, together with our earlier discovery of the importance of H_2O_2 production in the defense response of citrus flavedo to postharvest pathogens, indicate that the yeast-induced oxidative response in fruit exocarp may be associated with the ability of specific yeast species to serve as biocontrol agents for the management of postharvest diseases.