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Relationship between H_2O_2-detoxification, tolerance to H_2O_2 and virulence of some phytopathogenic bacteria

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ABSTRACT We studied correlation of catalase activity, tolerance to H_2O_2 and virulence of some phytopathogenic bacteria. *Pseudomonas syringae* pv. *tabaci* is a specific pathogen of tobacco. Other pathogenic bacteria, such as *Erwinia carotovora*, *Erwinia chrysanthemi*, *Agrobacterium tumefaciens* can infect a broad spectrum of plant species and there is no plant cultivar resistance against them. We have expected that these bacteria can protect themselves against the unspecific defence actions of plants which involve the accumulation of reactive oxygen species. We demonstrated a high level of catalase activity in a virulent strain of *Erwinia chrysanthemi*, as compared to a less virulent one. However, we did not find clear correlation between virulence levels and H_2O_2-detoxifying activity when we examined different bacterial species.
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KEY WORDS

virulence of bacteria
tolerance to H_2O_2
detoxification of H_2O_2

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), are generated in plants against the invading microorganisms. ROS provide toxicity to important macromolecules of pathogens as well as to the host cells often inducing rapid cell death (hypersensitive response). Previous reports have demonstrated that the extracellular polysaccharide (EPS) barrier against the ROS (Király et al. 1997) and the ROS-detoxifying mechanisms (Zhang et al. 2004) of phytopathogenic microorganisms may play important roles in their virulence when they defend themselves against the toxic effects of ROS produced by host plant cells.

Some phytopathogenic bacteria, such as *Erwinia carotovora*, *Erwinia chrysanthemi*, *Agrobacterium tumefaciens,* can successfully spread and multiply in many plant species and no specific resistance genes are available against them. One possible reason for their successful growth may be resulted from their elevated antioxidant defence capacity against the ROS produced in inoculated plant tissues. Therefore, we compared the H_2O_2-detoxifying activity and tolerance to H_2O_2 of these bacteria and *Pseudomonas syringae* pv. *tabaci*, a specific phytopathogenic bacterium which may induce resistance response in many plant hosts. We speculated that the limited success of *Pseudomonas syringae* pv. *tabaci* in resistant plant cultivars is due to a limited antioxidant activity of these bacterial cells.

Materials and Methods

Pathogens

Pseudomonas syringae pv. *tabaci* was provided by P. Ott (Plant Protection Institute, Hungarian Academy of Sciences, Budapest). *Agrobacterium tumefaciens* C58 was provided by S. Süle (Plant Protection Institute, Hungarian Academy of Sciences, Budapest). Other phytopathogenic bacteria, such as *Erwinia carotovora* 426 and *Erwinia chrysanthemi* 1679 and 1839 were isolated by M. Hevesy (Corvinus University, Budapest). Bacterial cultures were grown in M9 minimal medium containing 0.2% galactose and 0.01% nicotinic acid.

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Tests of tolerance to H_2O_2

Optical density of bacterial cultures was recorded by a photometer at 600 nm. Bacterial cells were grown in a freshly prepared medium for 24 hours before the experiment. Then the cultures were diluted to 0.02 OD 600 when we added the appropriate amount of H_2O_2 to the cultures. Then OD 600 of the cultures was detected in every 2 hours for 80 hours.

Catalase activity

The decrease of H_2O_2 levels was detected at 240 nm with a spectrophotometer (Shimadzu, Japan) in a quartz cuvette containing 2 mM H_2O_2 and 10^6 living bacterial cells from cultures of the same proliferation state in 2 ml 0.1 M sodium phosphate buffer (pH 6.0).

Results and Discussion

Erwinia chrysanthemi isolate 1839 is highly virulent, because it can cause soft rot on potato tuber slices in 24 hours. However, isolate 1679 can grow on potato tuber much more slowly, and causes typical symptoms in 4 days. This latter isolate is less virulent and shows low level of catalase activity, as compared to the virulent isolate 1839 (Fig. 1). Catalase activity of *Erwinia carotovora* was similar to the activity of

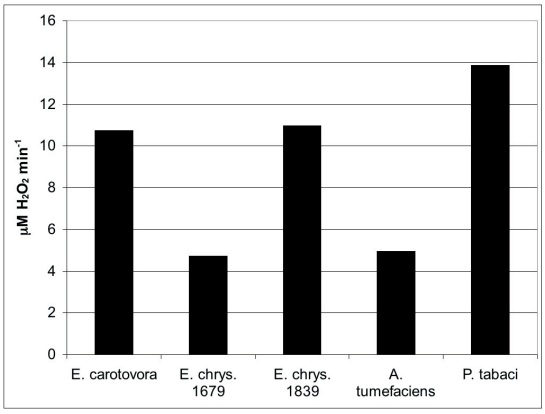

Figure 1. H_2O_2-detoxifying activity of living bacterial cells.

the virulent isolate of *Erwinia chrysanthemi* 1839 and it could cause soft rot in 24 hours on potato tuber slices.

Interestingly, the virulent strain of *Erwinia chrysanthemi* could tolerate only 0.25 mM H_2O_2 but the less virulent strain tolerated twofold higher level (0.50 mM) of hydrogen peroxide. The non-host-specific *Agrobacterium tumefaciens* and the host-specific *Pseudomonas syringae* pv. *tabaci* tolerated similar levels (0.50 mM) of H_2O_2. In contrast, *Erwinia carotovora* tolerated only 0.125 mM H_2O_2.

On the basis of the multiplication of phytopathogenic bacteria in the H_2O_2-containing medium one can conclude that the antioxidative defence capacity of bacterial cells does not always correlate with their tolerance to H_2O_2 and to their virulence. It is suggested that the frequency of producing new bacterial generations may modify their tolerance to H_2O_2.

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