Survival of *Pantoea ananatis*, causal agent of maize white spot disease in crop debris

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**ABSTRACT**

Maize white spot, caused by the bacterium *Pantoea ananatis*, is one of the most detrimental diseases of maize in Brazil and has contributed to significant yield reduction. In this study, *P. ananatis* isolates were recovered from maize white spot lesions, healthy leaves, corn crop residues and *Digitaria horizontalis*. All the bacterial isolates were identified and characterized by morphological traits analysis, acid production from sorbitol and glycerol, ice nucleation activity, metabolic fingerprint similarities and molecular analysis by Polymerase Chain Reaction with species-specific primers for *P. ananatis* (ANAF/ANAR). Results showed that isolates recovered from maize white spot lesions were similar to those isolates collected from other sources. It suggests that corn crop residues, *D. horizontalis* and healthy leaf surface of the corn plant itself, could be a source of survival for *P. ananatis*.

**Key words:** epiphytic bacteria, *Digitaria horizontalis*, metabolic fingerprint, ice-nucleation, corn crop residues.
The bacterial isolates showing similar morphological characteristics to \( P. \ ananatis \) were characterized by Gram staining, sorbitol/glycerol test (Schaad et al., 2001). The expression ice nucleation activity (INA) was also evaluated according protocol defined in Romeiro (2001). The bacterial isolates were incubated in 5 mL of TSB medium at 30 °C for 24 h with agitation (60 rpm). Subsequently samples were mixed by vortex to homogenize the samples and 100 μl were added to 1 mL of sterilized distilled water (cold at -10 °C), to evaluate ice formation. Negative controls were 100 μl of TSB bacteria free medium.

The metabolic fingerprint of isolates from lesions and from crop debris was performed using the biolog identification system (BIOLOG GN2 MicroPlate, Hayward, CA, USA). The metabolic patterns were read and identified using the MicroLog™ software. A similarity matrix among isolates was constructed using the Jaccard coefficient and the UPGMA method (Unweight Pair Group Method with Arithmetic Average) with NTSYS-Pc software (Applied Biostatistics Inc., NY, US).

For molecular analyses, the genomic DNA was purified according Gürtler and Stanisich (1996). The identity of the isolates was determined by partial analyses of the 16S rRNA gene or the species-specific pair of primers designed for \( P. \ ananatis \): ANAF (forward) (5'-CGTGAAAACCTACCGTGTTTG-3') and ANAR (reverse) (5'-TGCCAGGGATCCACCGTGTACCGT-3') (Figueiredo and Paccola-Meirelles, 2012). FTA cards (Whatman Inc., Clifton, USA) with preserved DNA from reference strains \( P. \ ananatis \) (PNA 08-2, PNA 97-5 and PNA 99-13) were used as positive controls. Specific PCR was performed to confirm \( P. \ ananatis \) isolates according Paccola-Meirelles et al. (2001). PCR was performed using 25 ng of genomic DNA as template. The reaction cocktail consisted of 2.5 μl 10x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 2.0 μM of each primer, 25 mM dNTP, 2.5 mM MgCl₂, and 1 U Taq DNA polymerase (Phonteutria, Belo Horizonte, Brazil) in a final volume of 25 μl. The amplification protocol consisted of previous denaturation step of 94 °C for 1 min followed by 30 cycles of amplification (94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min) with a final extension step of 72 °C for 10 min. PCR was performed in a model PTC-100 thermocycler machine (MJ Research, MS, USA). Amplicons were analyzed by horizontal gel electrophoresis at 6 V/cm² in 1.0% agarose gel (wt/v) in 1x TAE buffer (0.04 M Tris-Acetate, 0.001 M EDTA, pH 8.0) and subsequent staining with EtBr (0.5 mg/L) and visualized under UV light.

A total of 8 isolates were recovered (3 isolates from lesions, 2 from crop debris, 2 from healthy leaves and 1 from \( D. \ horizon-talis \)). All of them were Gram negative with colony morphology similar to \( P. \ ananatis \) and yellow pigmentation. All isolates were confirmed as \( P. \ ananatis \) species by PCR using the species-specific primers ANAF/ANAR. Ice nucleation and sorbitol/glicerol tests are summarized in Table 1, showing that crop debris isolates maintain the ice nucleation activity 60 days post-harvesting corn grains. Metabolic fingerprinting of all isolates was obtained and the similarity dendogram is shown in Figure 2. Isolates 1 and 3 were the most divergent and they were both collected from lesions (0.53 coefficient). Isolates from crop debris were included in a cluster that grouped one isolate from lesions (isolate 2) and other from healthy leaves (isolate 7) with 0.89 similarity coeffi-

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**Figure 1.** Maize white spot (MWS) symptoms. A) maize plants severely attacked by MWS; B) detail of the symptoms of MWS on maize leaf.

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D. horizontalis isolate (8) was clustered with 0.71 coefficient along with isolates 2, 4, 5, 6 and 7.

In this study the majority of isolates were positive to ice-nucleation, including those recovered from crop debris. This feature allows the bacterium to invade the host tissue more efficiently (Lindow et al., 1978) causing important agronomic and economical problems. In addition, the metabolic pattern of isolates recovered from crop debris were similar to those recovered directly from lesions or from healthy leaves, suggesting that, in crop debris, these bacteria could act similarly to epiphytic isolates, being a source of inoculum for further infections (Escanferla et al., 2006). The recovery of viable and culturable forms of P. ananatis from crop debris 60 days after harvest supports the hypothesis that the pathogen can survive under adverse conditions, minimizing its nutritional requirements (Wilson et al., 1999). Results from this study have contributed to increase knowledge on P. ananatis survival, which has also been reported to survive in other species of Digitaria spp. (Gitaitis et al., 2002).

In conclusion, P. ananatis may survive as epiphytic in the leaves of healthy maize plants, non-host plants and in crop debris, and possibly multiply there. Thus, knowledge on how this pathogen’s survival will help to improve the control of this important and serious foliar disease in maize.

Table 1. Ice nucleation activity and sorbitol/glicerol tests of isolates recovered from lesions, corn crop debris, maize healthy leaves and Digitaria horizontalis.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Ice Nucleation Activity</th>
<th>Acid production from Sorbitol/Glicerol</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>3</td>
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<td>6</td>
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<td>+/-</td>
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<tr>
<td>7</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Figure 2. Cladogram tree among Pantoea ananatis isolates applying the Jaccard coefficient of similarity and the UPGMA method using NTSYS-Pc software.

REFERENCES


Kido K, Adachi R, Hasegawa M, Yano K, Hikichi Y, Takeuchi...


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