

Title Development and application of a SCAR marker to monitor and quantify populations of the postharvest biocontrol agent *Pantoea agglomerans* CPA-2

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Citation Postharvest Biology and Technology, Volume 47, Issue 3, March 2008, Pages 422-428

Keywords Biological control; Citrus; Formulate cells; *Penicillium digitatum*; *Penicillium italicum*; Postharvest

Abstract

Pantoea agglomerans CPA-2 is an effective biocontrol agent of postharvest diseases of citrus and pome fruit. A monitoring technique was developed for its identification and to quantify its populations. The methodology used consisted of (i) searching for a semi-selective medium, (ii) identification of molecular markers and (iii) monitoring population dynamics in a commercial trial. As a semi-selective medium, Malonate Broth Agar supplemented with tetracycline hydroxychloride and incubation at high temperature (max. of 40 °C) facilitated the selective recovery of *P. agglomerans* CPA-2 colonies. The RAPD technique was applied to a collection of 13 strains of *P. agglomerans*, including CPA-2. Among the 12 primers tested, OPL-11 amplified a fragment (about 720 bp) specific to strain CPA-2. On the basis of this fragment, two SCAR markers were amplified using a primer pair derived from OPL-11 elongation. A first SCAR marker of 720 bp was specifically amplified for the strain CPA-2 and a second one of 270 bp was obtained for all *P. agglomerans* strains tested, including CPA-2. Commercial trials demonstrated a significant reduction of decay with the treatment of formulated cells of *P. agglomerans* CPA-2. Population dynamics of CPA-2 in commercial trials were determined on fruit surfaces and in the environment using both the classical plating technique and PCR with SCAR primers. In general, no significant differences were observed between results obtained from the two methods. On fruit surfaces, 1 day after CPA-2 applied its population by classical methods was 4.37×10^6 cfu wound⁻¹ and at the end of the experiment the population increased to 5.8×10^5 cfu wound⁻¹. The percentages of colonies identified as *P. agglomerans* CPA-2 at these sampling times using SCAR primers were 90 and 95%, respectively. Population dynamics in the environment to evaluate the environmental fate of *P. agglomerans* CPA-2 showed that it has a limited persistence and limited capacity for dispersion.