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PA 20, a semi-selective medium for isolation and enumeration of *Pantoea ananatis*

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Abstract

A semi-selective medium, PA 20, was developed for the isolation and enumeration of *Pantoea ananatis* from plant material, specifically from onion seed. The medium has a pH of 8.0 and contains $NH_4H_2PO_4$, K_2HPO_4, magnesium sulphate, NaCl, D (+) arabitol, crystal violet, bromothymol blue and thallium nitrate. All *P. ananatis* strains from a variety of hosts produced characteristic yellow colonies in 6–7 days at 25 °C. Plating efficiencies on PA 20 in comparison to nutrient agar ranged from 92 to 112%. Recovery from naturally infested and artificially contaminated onion seed was high, with an almost total reduction of saprophytes.

Keywords: PA 20 medium; *Pantoea ananatis*; Seed; Selective isolation

1. Introduction

Pantoea ananatis is a pathogen causing diseases in a number of economically important plants including onion (Gitaitis and Gay, 1997; Schwartz and Otto, 2000), *Eucalyptus (*Coutinho et al., 2002), corn (Paccola-Meirelles et al., 2001), melons (Bruton et al., 1986; Wells et al., 1987), Sudan grass (Azad et al., 2000), rice (Azegami et al., 1983) and pineapple (Serrano, 1928).

P. ananatis is both seed-borne and seed-transmitted in Sudan grass (Azad et al., 2000), rice (Tabei et al., 1988) and onions (Walcott et al., 2002). The disease of onion, caused by *P. ananatis*, was named center rot. Onion seed associated with the first outbreak of center rot in Georgia, USA (Gitaitis and Gay, 1997), was produced in South Africa and it was suspected that the bacterium was introduced into that country on infected seed lots (Walcott et al., 2002). In South Africa, efforts to control the quality of commercially produced onion

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seed mainly focus on the detection of fungal pathogens, and little is known about potential bacterial pathogens associated with these seeds. Nutrient Agar (NA) and yeast extract dextrose calcium carbonate (YDC) (Wilson et al., 1967) are the common growth media used to isolate *P. ananatis* from plant material and seed (Azad et al., 2000; Coutinho et al., 2002; Walcott et al., 2002). These media, however, are non-selective and many other organisms present as saprophytes or endophytes in plant material and in seed may hamper the detection of the target pathogen. In this paper, we describe a semi-selective medium, PA 20, which suppresses growth of many saprophytic microorganisms and serves as a suitable medium for growth and enumeration of *P. ananatis*. The medium was specifically developed to detect this pathogen on onion seed.

2. Materials and methods

2.1. Bacterial strains

Bacterial strains used in this study are listed in Table 1. Stock cultures of all isolates were main-

Table 1
Bacterial strains[a] used in this study, their growth on PA 20 medium and their pathogenicity to onion (*Allium cepa*) cv. Granex 33

Bacterium and strain number	Host	Growth on PA 20[b]	Pathogenicity to onion in a stub-inoculation test[c]
P. ananatis			
LMG 2665^T	*Ananas cosmosus*	+	−
LMG 2676	*Puccinia graminis*	+	−
LMG 20103, 20104	*Eucalyptus*	+	−,−
ATCC 35400	*Cucumis melo*	+	−
ATCC BAA 515	*Allium cepa*	+	−
Blackshank 15, 24, 30	*Allium cepa*	+	
Hort. Hill 7, 24, 31, 32			−,+,+,+
Pans 99-8, 99-23, 2002-2			−,−,+
BD 321, 322, 323, 234, 325, 326, 327, 328, 329	*Allium cepa*	+	all −
BD 377, 381, 383, 386, 387, 388, 390, 392, 393	*Allium cepa*	+	all +
P. agglomerans			
LMG 1286^T	knee laceration	−	−
SUH 2	*Allium cepa*	−	+
DAR 49828	*Allium cepa*	−	−
P. stewartii subsp. *indologenes*			
LMG 2632^T	*Setaria italica*	+[d]	−
LMG 2671	*Ananas cosmosus*	+[d]	−
Pseudomonas syringae	*Allium cepa*	−	not tested
Pectobacterium carotovorum subsp. *carotovorum*	*Solanum tuberosum*	−	not tested
Xanthomonas campestris pv. *alli*			
BD 142, 143, 211	*Allium cepa*	−	not tested
Saprophytes, 22 isolates	*Allium cepa* seed	−	all −

[a] LMG: BCCM/LMG Culture Collection, Universiteit Gent, Belgium; ATCC: American Type Culture Collection, Manassas, VA; DAR: Australian Collection of Plant Pathogenic Bacteria, Orange; BD: Plant Pathogenic and Plant Protecting Bacteria (PPPPB), ARC-PPRI, South Africa; Blackshank, Hort. Hill and Pans: R. Walcott, Department of Plant Pathology, University of Georgia, Athens; saprophytes from onion seed: this study.

[b] Growth on PA 20 medium from suspension of cells plated for isolated colonies.

[c] A sterile needle was dipped into the bacterial colony on NA (24–48 h growth) and then the needle was inserted under the epidermis of an onion leaf; results recorded after one week; pathogenic (+), non-pathogenic (−), not tested.

[d] Grew on the medium but colony morphology was different from *P. ananatis*.