Production of Monospecific Antiserum Against the Blood Disease Bacterium Affecting Banana and Plantain

B. Baharuddin, K. Rudolph, and F. Niepold

First and second authors: Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Grisebachstrasse 6, 37077 Göttingen, Germany; and third author: Biologische Bundesanstalt für Land- und Forstwirschaft, Institut für Pflanzenschutz in Ackerbau und Grünland, Messeweg 11/12, D-38104 Braunschweig, Germany.

Permanent address of B. Baharuddin: Department of Plant Pathology and Plant Protection, Hasanuddin University, 90245 Ujung Pandang, Indonesia.

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ABSTRACT

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Proteins from the blood disease bacterium (BDB) affecting banana and plantain and proteins from other bacterial species were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blotted onto nitrocellulose membrane, and probed with polyclonal antibodies directed against BDB. A 33.5-kDa protein band was characteristic for all virulent strains of BDB examined. Monospecific antibodies obtained against this protein by affinity purification were specific for BDB. A second polyclonal antibody was raised against the gel-purified 33-kDa protein in a rabbit. The serum was termed monospecific antiserum (MSA) and was used in subsequent experiments. The MSA reacted specifically with all virulent strains of BDB in agglutination tests and colony blot tests but not or only weakly with *Pseudomonas solanacearum, P. syzygii,* and other bacterial species. The specificity of the MSA was confirmed in studies with banana plant extracts. BDB could be detected in all infected plants 3–33 days after inoculation, whereas plants inoculated with *P. solanacearum* race 2 did not react with the MSA on immunocolony blots.

Additional keywords: *Pseudomonas* spp., serology, wilt disease.

The "blood disease" of banana and plantain, caused by a nonfluorescent pseudomonad, was first reported on the islands of Sulawesi and Selayar, Indonesia, in the 1920s (8). The name "blood disease" was given by the natives because of the white to reddish-brown bacterial ooze exuding from cut pseudostems or fruits. The characteristic symptoms, systemic and lethal wilt, leaf yellowing, followed by necrosis, collapse, and discoloration of fruits and vascular tissues, appeared to be very similar to moko disease caused by *Pseudomonas solanacearum* race 2 (2,6). Recently, taxonomic studies on the bacterial pathogen have been carried out. Several properties indicate that the blood disease bacterium (BDB) is closely related to *P. solanacearum* but distinct in host-range and nutritional characteristics (2,6). It also was possible to distinguish BDB from *P. solanacearum* by fatty acid (B. Baharuddin, J. Janse, and K. Rudolph, *unpublished data*) and restriction fragment length polymorphism analyses (4). Almost all of the present banana and plantain cultivars are susceptible to this disease (2). Although the disease has become the subject of a quarantine order since 1921, the disease is currently found in other islands in Indonesia, such as Kalimantan, Molucca, Java, Sumatra, and Irian Jaya. During recent years, the disease has caused severe losses and become a limiting factor in the cultivation of banana and plantain in Indonesia (2,10).

A method for sensitive and quick diagnosis of the disease is a prerequisite for controlling the disease by plant hygienic measures. Until now, specific antiserum has not been available for detection of the pathogen. Polyclonal antisera against whole bacterial cells, which were tested earlier, showed extensive cross-reactions with *P. solanacearum* strains that cause wilt in a wide variety of plant species and with *P. syzygii*, the incitant of Sumatra wilt disease on clove (1,7). Our goal was to develop a specific antiserum against BDB affecting banana and plantain.

MATERIALS AND METHODS

General procedure. The general procedure for production of monospecific antiserum (MSA) against BDB is shown in Figure 1.

Origin and growth of bacteria. Strains of BDB were obtained mainly from diseased banana plants in Sulawesi, Java, Moluccas, and Kalimantan; some strains were provided by S. J. Eden-Green, Rothamsted Experiment Station, Hertfordshire, England. Strains of *P. solanacearum* were obtained from the Institut für Pflanzenpathologie und Pflanzenschutz der Universität's (Göttingen, Germany) collection (Göttinger Sammlung phytopathogener Bakterien = GSPB); from ICMP, Auckland, New Zealand (International Collection of Micro-organisms from Plants); NCPPB, Harpendon, England (National Collection of Plant Pathogenic Bacteria); and RCC, Rothamsted Experiment Station, England (Rothamsted Culture Collection) (Table 1). The bacteria were grown and maintained on Kelman's tetrazolium medium (16), casein peptone glucose (CPG) agar, or on nutrient dextrose agar (16) and incubated at 29 C for 2–3 days after transfer.

Production of polyclonal antiserum directed against BDB. Whole cells of BDB (strain P02) were used to generate polyclonal antiserum (AS 389) and were prepared for immunization as described earlier (13).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). To prepare bacterial proteins, the bacteria were grown in Rhodes' liquid medium (15) at 30 C for 24 h and sedimented in Eppendorf tubes by centrifugation (11,600 *g* for 4 min). The cell density was about 10^9 cells per milliliter. Lysates of bacterial cells were prepared by treatment with lysozyme (2 mg/ml in 50 mM glucose, 10 mM EDTA, 25 mM Tris/HCl, pH 8) and 140 μl of bromophenol blue (BPB) solution (200 mM Tris/HCl, pH 8; 0.5 M saccharose; 0.1% BPB; 5 mM EDTA; 5% β-mercaptoethanol; 3% SDS), and proteins were denatured by boiling in water for 7 min (5). The bacterial proteins were separated by SDS-PAGE (17).

Western blotting. The proteins were transferred by electroblotting onto nitrocellulose membrane filters (NC type BA 85, Schleicher & Schuell, Dassel, Germany; 0.45 μm) as described by Shah and Stegemann (17).

Serological reactions. The membranes containing bacterial proteins were blocked with 3% BSA in TN buffer (10 mM Tris/HCl, 150 mM NaCl, pH 8.0) for at least 1 h. The procedure for incubation of the NC membranes with antiserum was adopted from Bjerrum and Heegard (3). The membranes were incubated with a 1:500 dilution of an antiserum generated against whole cells of BDB (AS 389). To prevent growth of contaminating microorganisms, NaN_3 was added at a final concentration of 0.025%. After incubation, the blots were washed three times with TNT (10 mM Tris/HCl, pH 8.0; 150 mM NaCl; 1% Triton X-100) for 20 min, and once with TN buffer for 10 min.

To detect antibodies bound to proteins of BDB, secondary antibodies (anti-rabbit IgG coupled with alkaline phosphatase, Sigma Chemical Co., St Louis) were applied. The incubation of the secondary antibodies was performed according to the supplier's instructions at a dilution of 1:1,000 in 1% BSA-TN buffer. After washing (three times for 10 min with 1% BSA-TN and once with distilled water for 20 min), color development of positive antibody reactions was accomplished with the dye Fast Red (Fast Red Salt TR and Naphthol-AS-MX phosphate, Sigma). The staining reaction was stopped by washing the blot with distilled water. Blots were air-dried, mounted onto cardboard, and stored at room temperature.

Determination of BDB-specific proteins. To determine molecular weight, the protein bands of all tested bacteria were compared with reference protein bands (Merck standard 4, Merck Co., Darmstadt, Germany) in Coomassie blue-stained gels. The proteins which were unique to BDB and which bound the antibodies in immunoblots were selected as specific antigens.

Elution of monospecific antibodies from a selected protein band. To obtain monospecific antibodies from the polyclonal antiserum, the protein extracts of BDB were separated by electrophoresis and electroblotted onto NC membranes using all available slots of the SDS-PAGE gel. After immunostaining the left and right borders of the membrane, the location of the selected protein band in the untreated middle part of the membrane was determined, using both stained borders as a marker. Finally, the membrane strip containing the protein was cut out.

TABLE 1. Reaction of antibodies against different strains of the blood disease bacterium (BDB), *Pseudomonas solanacearum*, and other bacteria in agglutination tests

Strain (GSPB)[a]	Infected cultivars	Geographic origin and source[b]	Reaction[c]			
			AS 389	AS 4990	MSA	K
BDB						
P02 (1845)	Musa ABB	S. Sulawesi	+++	±	++	−
H22a (1891)	Musa AAA	W. Java	+++	nt	++	−
T440 (1793)	Musa ABB	N. Sulawesi, Eden-Green	++	nt	+	−
H02 (1843)	Musa AAB	S. Sulawesi	++	++	++	−
M01b (1896)	Musa ABB	Molucca	++	nt	++	−
M01a (1895)	Musa ABB	Molucca	+++	+	++	−
H11 (1890)	Musa ABB	S. Sulawesi	+	+	±	−
H08 (1853)	Musa ABB	S. Sulawesi	++	nt	+	−
B2e (2138)	Musa ABB	E. Kalimantan	++	++	++	−
P. solanacearum						
523	Bean	Mauritius, GSPB	++	+++	−	−
2015	Tomato	USA, RCC	+	++	−	−
2014	Tobacco	Australia, NCPPB	+	+++	−	−
2111	Capsicum	Indonesia, RCC	+++	++	−	−
2116	Ginger	Indonesia, RCC	+++	+++	−	−
2124	Potato	Indonesia, RCC	+++	+++	−	−
2118	Ricinus	Indonesia, RCC	+++	++	−	−
1510	Musa AAB	Costa Rica, GSPB	−	+++	−	−
2123	Musa AAA	Philippines, RCC	++	++	−	−
2115	Heliconia	Costa Rica, RCC	+++	+++	−	−
2125	Musa ABB	Peru, RCC	++	++	−	−
2134	Strelitzia	Taiwan, RCC	+++	+++	−	−
2130	Mulberry	China, RCC	+++	+++	−	−
1960	*S. dulcamara*	Sweden, NCPPB	−	++	±	−
P. syzygii						
2088	Clove	Sumatra, RCC	+++	+++	−	−
2089	Clove	Java, RCC	+++	+++	±	−
X. c. celebensis[d]						
1783	Banana	NCPPB	−	−	−	−
1630	Banana	ICMP	−	±	−	−
P. flourescens (G1)		GSPB no. 1714	−	−	−	−
P. pickettii (1625)		S. Seal no. 1625	−	−	−	−
P. s. atrofaciens (1727)[e]	Wheat	Germany, GSPB	±	−	−	−
P. s. coriandricola (1818)	Coriander	Germany, GSPB	±	−	−	−
P. s. glycinea (2033)	Soybean	Germany, GSPB	−	−	−	−
P. s. lachrymans (1480)	Cucumber	Germany, GSPB	−	−	−	−
P. s. phaseolicola (1715)	Bushbean	Germany, GSPB	−	−	−	−
P. s. tomato (478)	Tomato	Greece, GSPB	±	−	−	−
P. viridiflava (1685)	Bushbean	Switzerland, GSPB	−	−	−	−
X. c. phaseoli (1241)	Bushbean	USA, GSPB	−	−	−	−

[a] GSPB: Göttinger Sammlung phytopathogener Bakterien Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Göttingen, Germany.
[b] S. J. Eden-Green, Rothamsted Experiment Station, Hertfordshire, England; NCPPB: Nat'l. Collect. Plant Path. Bact., Harpendon, England; RCC: Rothamsted Cult. Collect., England; ICMP: Int'l. Collect. Micro-org. Plants, Aukland, New Zealand; BDB strains were from Indonesia.
[c] AS 389: Antiserum raised from rabbit against whole cell of BDB; AS 4990: antiserum raised from goat against a mixture of races 1, 2, and 3 of *P. solanacearum*; MSA: monospecific antiserum; and K: control. nt = not tested.
[d] *X. c.* = *Xanthomonas campestris.*
[e] *P. s.* = *Pseudomonas syringae.*

The selected NC strip was blocked with 3% BSA-TN buffer and incubated at room temperature for 2 h with the polyclonal antiserum solution (1:500) directed against whole BDB cells. NaN_3 was added to the antiserum solution to a final concentration of 0.025% to inhibit the growth of contaminating microorganisms. The blot was washed with TNT (three times for 10 min) and TN (once for 10 min) to remove the nonspecific antibodies. The specific antibodies bound to the protein were released from the NC strip by a pH shift to acidity (14). This was achieved by immersing the NC strip in 0.2 M glycine HCl, pH 2.8, for a few seconds. The solution containing the released antibodies was immediately placed on ice and neutralized to pH 7.0 with 1 M NaOH. The protein strip, stored in TN buffer, could be re-used up to 10 times for repeated antibody purification. To reduce the buffer volume containing the monospecific antibodies, the solution was dihydrated in powdered sucrose.

Use of the selected protein band from BDB to generate MSA in rabbits. To generate MSA in rabbits against the desired protein from NC membrane, the protein containing the NC strip was cut into small pieces and dissolved in dimethyl sulfoxide (DMSO) until the solution was clear and could be pipetted. Addition of an equal volume of 10 mM phosphate buffer in 0.15 M NaCl (pH 7.2) to the DMSO-NC solution precipitated the protein containing NC. After centrifugation, the NC protein pellet was washed twice with 10 mM phosphate buffer (pH 7.2) containing 0.15 M NaCl, resuspended in 1 ml of the same buffer, and mixed with 1 ml of Freund's complete adjuvant (Sigma) (G. Adam, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, *personal communication*). The suspension was injected subcutaneously into rabbits (repeated twice at 7- to 10-day intervals). Extraction and purification of the polyclonal antibodies directed against the specific protein were performed as described earlier (11,13).

Agglutination tests. Agglutination with bacterial cells was performed on slides as described by Niepold and Huber (13).

Immunocolony blot tests with extracts from infected banana plants. The immunocolony blot followed the method briefly reported previously (1,9). Tissue-cultured Indonesian banana, ABB cultivar Pisang Kepok, was used for inoculation. Three months after transplanting, the plants were stem-inoculated with a syringe containing about 10^7 cfu of the BDB strain P02 and *P. solanacearum* race 2 strain 1859 per milliliter, respectively. Two or three sections were serially cut from the pseudostem 3, 6, 9, and 12–33 days after inoculation. Each sample with two or three repititions consisting of a 1-cm-long piece of the pseudostem was separately macerated and serially diluted in 0.01 M $MgSO_4$ and then streaked onto CPG medium. The plates were incubated at 29 C for 2–3 days.

Plates containing 30–50 bacterial colonies were selected for subsequent experiments. An NC membrane was pressed onto an agar plate containing bacterial colonies with a glass spatula. The NC membrane was removed, and a second membrane was placed on the colony-containing side of the first membrane. Both membranes were pressed between two glass petri dishes. The second membrane was incubated 5 min in SDS solution and washed three times with H_2O and once with TN buffer. The second membrane was necessary to obtain distinctive colony patterns. The membrane was blocked with 3% BSA-TN for 2 h and incubated with MSA overnight. The blots were washed three times for 10 min with TNT and once for 10 min with TN. Anti-rabbit IgG coupled with alkaline phosphatase (1:1,000 in 1% BSA-TN) was used as secondary antibody by incubating for 2 h. The blots were washed three times with 1% BSA-TN and once for 10 min with TN. The bound MSA was visualized by staining with Fast Red.

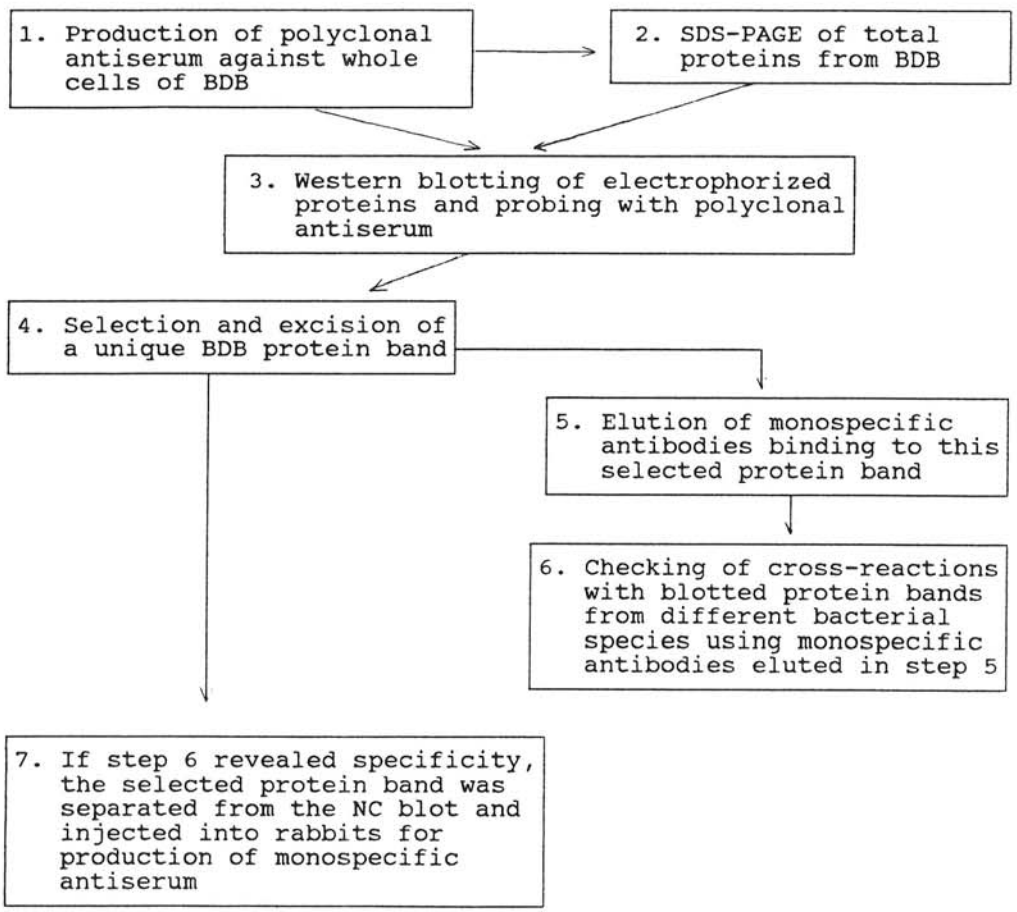

Fig. 1. Flow chart for production of monospecific antiserum against blood disease bacterium (BDB). SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

RESULTS

A comparison of bacterial protein bands by SDS-PAGE with reference protein bands (Merck standard 4) revealed a 33.5-kDa protein that was typical for virulent strains of BDB. It was not detected in protein profiles of the *P. solanacearum* or *P. fluorescens* strains tested. In avirulent or weakly virulent strains of BDB, this protein band was not distinctly visible. From the SDS gel the proteins were transferred onto NC membranes and probed with antiserum raised against BDB by immunoblotting (Fig. 2).

To separate antibodies from the complex polyclonal antiserum specific for the 33.5-kDa protein band, the selected protein band was cut out and incubated with the polyclonal antiserum. The antibodies eluted from this protein band showed a distinct reaction only with the 33.5-kDa protein of BDB, not with other protein bands from either BDB or *P. solanacearum* (Fig. 3). A weak cross-reaction was observed with one protein band of *P. solanacearum* but at a different position than the 33.5-kDa protein band of BDB. The weakly virulent strain H08 of BDB showed a faint reaction at the same position as the virulent strain P02, whereas the avirulent BDB strain H11 and *P. fluorescens* did not react at all with the monospecific antibodies. Therefore, the 33.5-kDa protein band was used as antigen to produce MSA for BDB.

The results of testing the antisera against *P. solanacearum*, BDB, and other bacteria in a microagglutination test are summarized in Table 1. The polyclonal antisera AS 389 and AS 4990 cross-reacted with BDB, *P. solanacearum*, and also with *P. syzygii*. However, the MSA raised against the 33.5-kDa protein band of BDB reacted only with BDB strains. Reaction with an avirulent

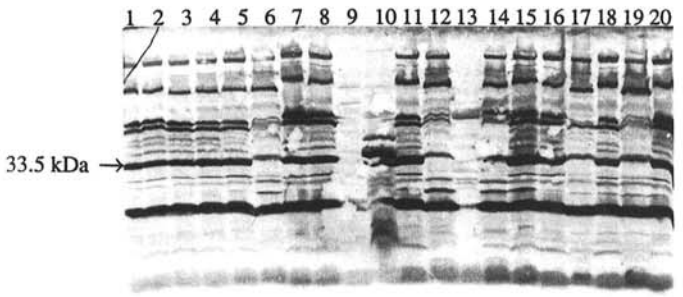

Fig. 2. Comparison of protein bands from blood disease bacterium (BDB), *Pseudomonas solanacearum*, and *P. fluorescens* blotted onto nitrocellulose membrane and probed with a polyclonal antiserum against BDB. Lanes 1–5, 7, 8, 11, 15, 16, 18, and 20: highly virulent strains of BDB, T394, M01b, H06b, T389, H02, H18, H03, H22a, M01a, T440, and T334, respectively. Lane 9: avirulent strain of BDB (H11). Lane 10: weakly virulent strain of BDB (H08). Lanes 6, 12, 17, and 19: *P. solanacearum* race 2, GSPB (Göttinger Sammlung phytopathogener Bakterien) 1513, 1859, 1508, and 1511, respectively. Lane 13: *P. fluorescens*. Lane 14: *P. solanacearum* race 1, strain GSPB 523.

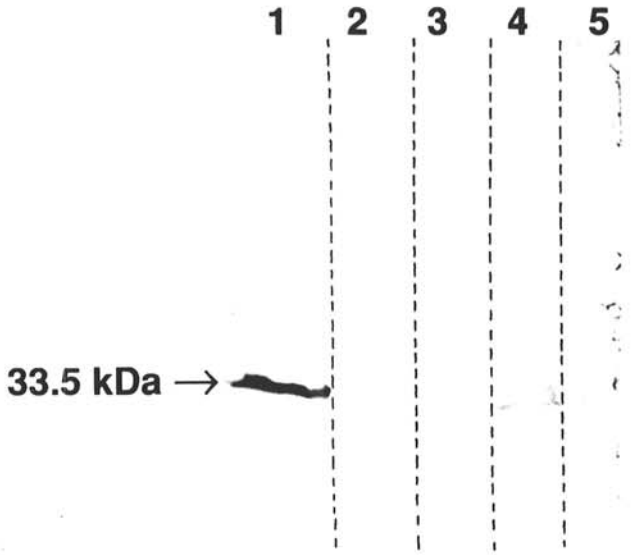

Fig. 3. Reaction of a protein blot of different bacterial strains with antibodies specific to the 33.5-kDa protein band of the blood disease bacterium (BDB) (i.e., monospecific antibodies). Lane 1: BDB, strain P02; lane 2: *P. solanacearum*, strain GSPB (Göttinger Sammlung phytopathogener Bakterien) 1513; lane 3: avirulent strain of BDB (H11); lane 4: weakly virulent strain of BDB (H08); and lane 5: *P. fluorescens*.

TABLE 2. Reaction of the monospecific antiserum against pure cultures of the blood disease bacterium (BDB) and *Pseudomonas solanacearum* in immunocolony blot tests

GSPB Strain no.[a]	Infected cultivars	Geographic origin and source[b]	Reaction[c] with MSA (1:750)
BDB			
1790	Musa AAA	W. Java, Eden-Green	++
1843	Musa AAB	S. Sulawesi	++
1844	Musa ABB	S. Sulawesi	++
1845	Musa ABB	S. Sulawesi	++
1891	Musa ABB	S. Sulawesi	++
1793	Musa ABB	N. Sulawesi	++
1895	Musa ABB	Molucca	++
1853	Musa ABB	S. Sulawesi	+
1855	Musa ABB	S. Sulawesi	++
2012	Musa AAA	W. Java, Eden-Green	++
P. solanacearum			
1958	Potato	Egypt, NCPPB, race 3 Bv. 2	−
1508	Musa ABB	Costa Rica, GSPB, SFR strain	−
1509	Musa ABB	Costa Rica, GSPB, SFR strain	−
1510	Musa AAB	Costa Rica, GSPB, B strain	−
1511	Musa AAB	Costa Rica, GSPB, B strain	−
1512	Musa AAA	Costa Rica, GSPB, A strain	−
1513	Musa AAA	Costa Rica, GSPB, A strain	−
1859	Musa AAA	Brazil, ICMP	±
1959	Eggplant	Egypt, NCPPB	−
2014	Tobacco	Australia, NCPPB	−
2015	Tomato	USA, RCC	−

[a] GSPB: Göttinger Sammlung phytopathogener Bakterien, Institut für Pflanzenpathologie und Pflanzenschutz der Universität, Göttingen, Germany.
[b] S. J. Eden-Green, Rothamsted Experiment Station, Hertfordshire, England; NCPPB: Nat'l. Collect. Plant Path. Bact., Harpendon, England; ICMP: Int'l Collect. Micro-org. Plants, Aukland, New Zealand; RCC: Rothamsted Cult. Collect., England; BDB strains were from Indonesia.
[c] MSA: Monospecific antiserum raised from rabbit against a 33.5-kDa protein band of BDB.

BDB strain (H11) was weak. MSA did not react with strains of *P. solanacearum* or other bacterial species and pathovars. Thus, BDB cells agglutinated well with MSA, whereas *P. solanacearum* showed no reaction.

The immunocolony blot test was used to identify BDB not only in pure bacterial cultures but also in infected banana plants. This method is more sensitive than the agglutination test. Thus, up to a dilution of 1:750, MSA reacted strongly with blotted colonies of BDB strain P02. Dilutions of 1:1,000 showed a moderate reaction, and those of 1:1,250 and 1:1,500 reacted weakly. Almost all strains of BDB from pure cultures reacted positively with the specific antibodies, only strain H08 (weakly virulent) reacted weakly. All strains of *P. solanacearum* reacted negatively except one (GSPB 1859) that showed a weak reaction (Table 2).

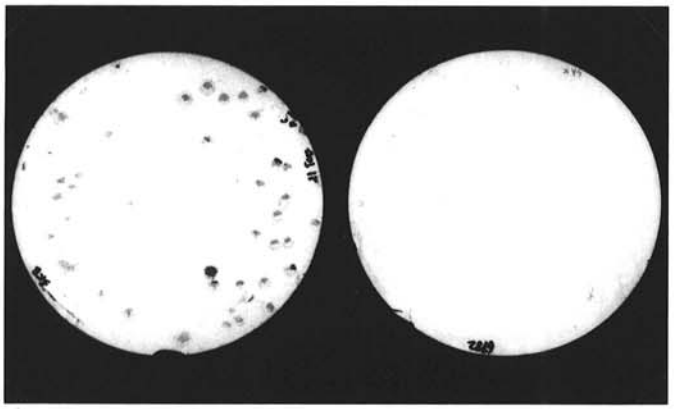

Fig. 4. Reaction of blood disease bacterium colonies, strain P02 (left), and *Pseudomonas solanacearum* colonies, strain GSPB (Göttinger Sammlung phytopathogener Bakterien) 1513 (right), blotted onto nitrocellulose membranes with the monospecific antiserum; the colonies resulted from extracts of banana plants 21 days after bacterial inoculation.

The specificity of the antibodies could be confirmed with colonies grown from banana plant extracts. Using the colony blot test, BDB was detected by the MSA and visualized as red spots on NC membranes, indicating a positive antibody reaction. In contrast, the MSA did not react with *P. solanacearum* (Fig. 4).

Figure 5 shows the number of BDB colonies detected on NC membranes in extractions from banana plants at different time intervals after inoculation. BDB were detected at all stages between 3 and 33 days after inoculation, although wilt symptoms started to appear 2 wk after inoculation. The detection level of BDB ranged between 10^4 and 10^9 cells per milliliter in these experiments. Compared to the immunocolony blots, higher numbers of colonies were always counted on agar plates, because bacterial contaminants were included. Also, isolations from *P. solanacearum*-infected banana plants resulted in high numbers of bacterial colonies on agar plates. In contrast, on NC membranes MSA-positive colonies were never seen in extracts from banana plants infected by *P. solanacearum*.

Principally, the same results regarding bacterial numbers on agar plates versus NC membranes were obtained independently at 11 sampling dates (Fig. 5). For both pathogens, maximum populations in planta were reached between 18 and 21 days after inoculation, with 9 to 10^8 cfu per milliliter for BDB and 1 to 10^9 cfu per milliliter for *P. solanacearum* when calculated from the numbers on agar plates. From 21 to 33 days after inoculations bacterial populations decreased steadily. This was probably due to a progressing discoloration and degeneration of the plant tissue accompanied by increased numbers of saprophytes.

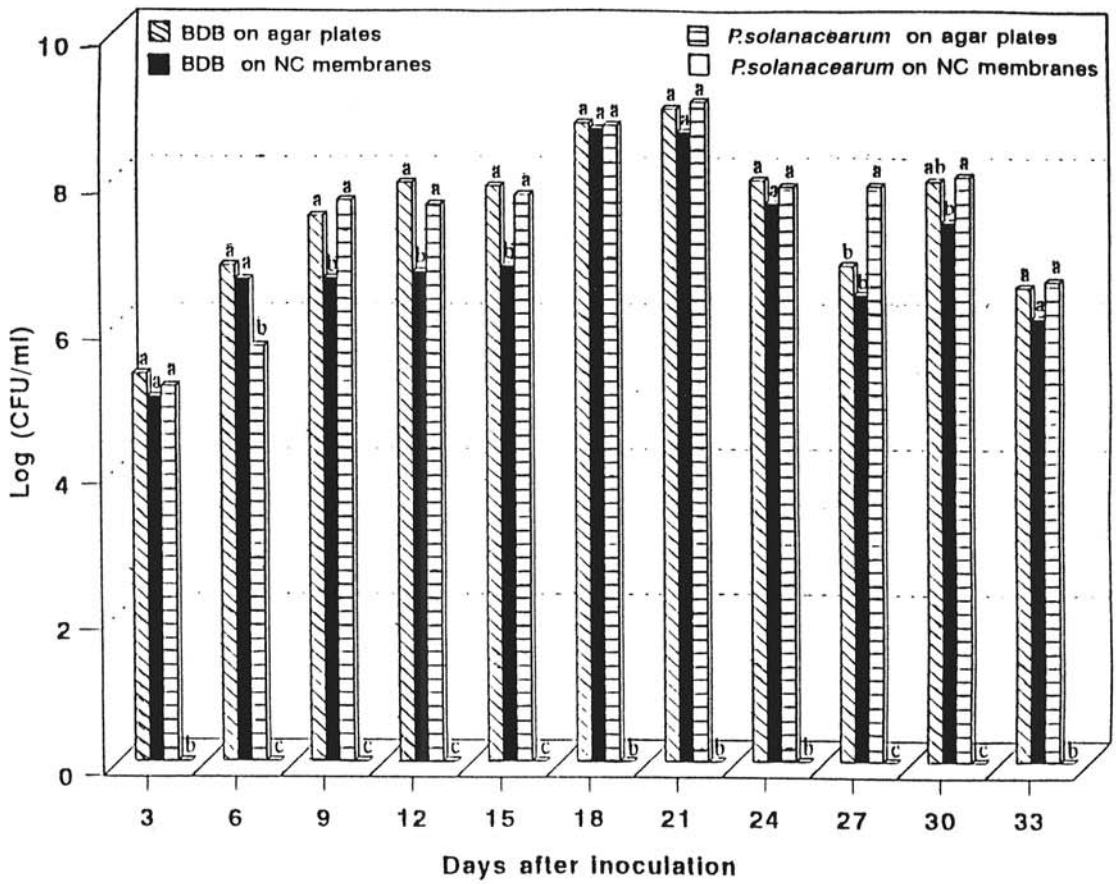

Fig. 5. Number of blood disease bacterium (BDB) (strain P02) and *Pseudomonas solanacearum* (strain GSPB [Göttinger Sammlung phytopathogener Bakterien] 1859) colonies on isolation plates compared with number of colonies detected by an immunocolony blot procedure using monospecific antiserum. The samples were taken from artificially stem-inoculated banana plants ABB cv. Pisang Kepok 3, 6, and 9–33 days after inoculation. Columns within each sampling date, capped with the same letters are not significantly different at the 1% level (Duncan's multiple range test).

DISCUSSION

The bacterial pathogen of BDB has not yet been fully characterized, but several properties distinguish this bacterium from *P. solanacearum*, which also causes wilt disease of banana and plantain. The two pathogens can be distinguished by carbohydrate-utilization patterns, using the Biolog system (19), and by nucleic acids studies (4,18).

Eden-Green et al (7) found that BDB can neither reduce nitrate nor hydrolyze gelatin. Other characteristics of BDB are: nonfluorescent; poly-β-hydroxybutyrate positive; mucoid, small round colonies with a red center on tetrazolium medium; oxidase positive; and HR positive (2,7). Numerical analysis of 78 phenotypic characters showed that BDB forms an intermediate cluster between *P. solanacearum* biovar 1 and *P. syzygii* (6).

Our studies showed that BDB also can be differentiated from related bacteria by serological methods, such as selecting a BDB-specific 33.5-kDa protein that can be used as antigen to produce specific polyclonal antiserum. In earlier experiments, this method was used to produce MSA against *P. syringae* pv. *syringae* (11,12). The 33.5-kDa protein band was distinct from other protein bands on an immunoblot and appeared clearly only in virulent strains of BDB. Evidently, this protein band is correlated with virulence of BDB, but it is unknown whether it plays a role in pathogenesis. Monospecific antibodies purified from a complex antiserum by an antigen affinity procedure reacted strongly only with the 33.5-kDa protein band on Western blots.

To examine specificity of the rabbit antiserum for BDB, we used two serological methods (agglutination and immunocolony blot tests). In both tests, the monospecific antibodies reacted solely with pure cultures of BDB strains, not with other bacterial species or pathovars. Obviously, the weak cross-reaction observed with a protein band of *P. solanacearum* after SDS-PAGE (Fig. 3) was not sufficient to result in a positive serological reaction with whole bacterial cells. In the immunocolony blot tests, MSA also detected BDB from artificially inoculated banana plants with high sensitivity, whereas extracts from banana plants inoculated with *P. solanacearum* did not react with the MSA.

The results indicate that the MSA can detect BDB in plants during the latent stage of disease. Whether it is possible to detect BDB from naturally infected plants or from insect vectors by this method has not been investigated. In comparison with enzyme-linked immunosorbent assay or immunofluorescence, the immunocolony blot test appears more advantageous, because this method does not require expensive laboratory equipment, such as a spectrophotometer or fluorescence microscope. Another advantage is that MSA can be produced at lower costs compared to monoclonal antibodies. Because MSA detects only one specific protein band, the sensitivity is not as high as that of conventional polyclonal antisera reacting with more than one antigen. Further studies are planned to test whether the MSA can be used successfully for diagnosis under field conditions.

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