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Rapid Identification of *Burkholderia pseudomallei* by Latex Agglutination Based on an Exopolysaccharide-Specific Monoclonal Antibody

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We recently identified a constitutively expressed exopolysaccharide of *Burkholderia pseudomallei* which is composed of a unique linear tetrasaccharide repeating unit consisting of three galactose residues and one 3-deoxy-D-*manno*-2-octulosonic acid residue. In this study we developed a latex agglutination test based on monoclonal antibody 3015, which is specific for this exopolysaccharide, and evaluated this test for rapid identification of *B. pseudomallei* grown on agar plates. All 74 environmental and clinical *B. pseudomallei* strains tested, originating from different areas of Southeast Asia, northern Australia, and Africa, showed a strong and specific agglutination. *B. pseudomallei*-like organisms and a variety of other bacteria did not react. In conclusion this monoclonal antibody-based test is a simple, rapid, and highly specific method for identifying *B. pseudomallei* culture isolates from different geographic areas.

Melioidosis is an infectious disease caused by the gram-negative rod *Burkholderia pseudomallei*. The disease is now recognized as an important public health problem in certain areas of the tropics (4). Identification of this species can be achieved by a combination of the commercial API 20 NE biochemical kit and a simple screening system involving Gram's stain, the oxidase reaction, typical growth characteristics, and resistance to certain antibiotics (5). However, these methods are relatively laborious, and classical identification seldom requires less than 48 h (11). The early diagnosis of *B. pseudomallei* is crucial for appropriate antibiotic treatment, and therefore suspicious colonies should be rapidly identified as soon as they are visible on the agar. Recently, a nonvirulent biotype of *B. pseudomallei* was defined by the ability to assimilate L-arabinose (8), but it could not be distinguished immunologically (12). It has been proposed that this L-arabinose-assimilating biotype should be reclassified as a *B. pseudomallei*-like species, termed *B. thailandensis* sp. nov (2).

We recently identified and purified a constitutively expressed species-specific exopolysaccharide of *B. pseudomallei* reactive with the monoclonal antibody (MAb) 3015 immunoglobulin G1 (IgG1) (10). This exopolysaccharide appeared to be a unique linear tetrasaccharide repeating unit consisting of three galactose residues and one 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) residue (6). In this study we developed a latex agglutination test based on the MAb 3015 IgG1 and evaluated this test for the rapid identification of *B. pseudomallei* culture isolates originating from different areas in Southeast Asia, Australia, and Africa.

Bacteria and culture conditions. All bacterial strains used in this study are listed in Table 1. The L-arabinose-nonassimilating (Ara^-) *B. pseudomallei* strains originating from Thailand were either clinical strains obtained from patients admitted with melioidosis to Sappasitprasong Hospital, Ubon Ratchatani, northeast Thailand, or soil isolates from the surrounding area (12). L-Arabinose-assimilating (Ara^+) *B. pseudomallei*-like strains were from the same environment (12). *B. pseudomallei* strains (Ara^-) originating from other countries were obtained either from the National Collection of Type Cultures (NCTC), United Kingdom, or from Tyron Pitt, Laboratory of Hospital Infection, Central Public Health Laboratory, London, United Kingdom. All bacterial strains were grown on Columbia agar for 24 h at 37°C.

ELISA. The production of MAb 3015 IgG1 and the enzyme-linked immunosorbent assay (ELISA) used in this study have previously been described (10). Briefly, single U-shaped wells of nonflexible polystyrol microtiter plates were coated with heat-treated *B. pseudomallei* and *B. pseudomallei*-like cells (2×10^8 cells/ml) for 2 h. After washing and blocking steps, MAb 3015 IgG1 containing hybridoma supernatant (5 μg/ml) was incubated for 2 h followed by the addition of biotin-labeled rabbit anti-mouse IgG1 (1:5,000 in buffer A-BSA; Zymed, San Francisco, Calif.) (buffer A-BSA is buffer A [0.01 M potassium phosphate buffer made isotonic with saline, pH 7.5] containing 1% [wt/vol] bovine serum albumin [BSA] and 0.1% [wt/vol] NaN_3) for 30 min. Microtiter plates were then developed with streptavidine coupled to β-galactosidase. 4-Methylumbelliferyl-β-D-galactopyranoside was used as substrate, and the fluorescent product was measured with a microplate fluorometer (Titertek Fluoroskan 2; Labsystems, Helsinki, Finland) with an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Fluorescence intensity is given as relative fluorescence units.

Preparation of the latex reagent. MAb 3015 containing hybridoma supernatant and a control IgG1 were concentrated by ultrafiltration with a ProVario 3 apparatus (Pall-Filtron, Karlstein, Germany) by using a membrane with a cutoff of 10 kDa and then purified by affinity chromatography on protein-G-Sepharose (Pharmacia, Uppsala, Sweden). Purified antibodies were diluted in buffer A. Latex particles (1.07 μm in diameter) supplied as a 10% (wt/vol) suspension (Sigma) were washed three times in distilled water and resuspended to the original volume. All washing steps were performed at 13,600 × *g* for 5

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TABLE 1. Reactivities of *B. pseudomallei* and non-*B. pseudomallei* isolates with the MAb 3015 IgG1-based latex test

Strain	Source	Origin	Latex test reaction[a]
B. pseudomallei strains			
NCTC 4845	Monkey	Singapore	+
NCTC 1688	Rat	Malaysia	+
NCTC 4846	Monkey	Singapore	+
NCTC 6700	Human	Malaysia	+
NCTC 7383	Human	Burma	+
NCTC 7431	Human	Unknown	+
NCTC 8016	Sheep	Australia	+
NCTC 10274	Human	Malaysia	+
NCTC 10276	Human	Bangladesh	+
NCTC 8707	ND[b]	Singapore	+
NCTC 8708	ND	Singapore	+
NCTC 11642	ND	Unknown	+
308A	Human	Thailand	+
309A	Human	Thailand	+
310B	Human	Thailand	+
312A	Human	Thailand	+
369A	Human	Thailand	+
388A	Human	Thailand	+
406B	Human	Thailand	+
423A	Human	Thailand	+
428D	Human	Thailand	+
430E	Human	Thailand	+
435A	Human	Thailand	+
448A	Human	Thailand	+
688A	Human	Thailand	+
708A	Human	Thailand	+
722A	Human	Thailand	+
778A	Human	Thailand	+
785A	Human	Thailand	+
843B	Human	Thailand	+
846A	Human	Thailand	+
854A	Human	Thailand	+
E08	Soil	Thailand	+
E202	Soil	Thailand	+
E203	Soil	Thailand	+
E208	Soil	Thailand	+
E210	Soil	Thailand	+
E212	Soil	Thailand	+
E213	Soil	Thailand	+
E219	Soil	Thailand	+
E220	Soil	Thailand	+
E235	Soil	Thailand	+
E237	Soil	Thailand	+
521	Human	Pakistan	+
MK441	Monkey	Philippines	+
MK452	Monkey	Philippines	+
MK453	Monkey	Philippines	+
M1900	Monkey	Philippines	+
M1831	Monkey	Indonesia	+
M1867	Monkey	Indonesia	+
Ramal	Human	Thailand	+
56-91	Camel	Chad	+
770429	Soil	Madagascar	+
P19535/91	Human	Singapore	+
Sar1	Human	Sarawak	+
6068	ND	Southeast Asia	+
T8 + 8	ND	Austria	+
501944	Monkey	Philippines	+
NT08	Soil	Niger	+
7605	Manure	France	+
Ducrete	Human	Vietnam	+
S1	Human	Singapore	+
S3	Human	Singapore	+
S6	Human	Singapore	+
S9	Human	Singapore	+
S12	Human	Singapore	+
S15	Human	Singapore	+
L19	ND	ND	+
L23	ND	ND	+
L47	ND	ND	+
L85	ND	ND	+
L87	ND	ND	+
L90	ND	ND	+
L91	ND	ND	+
B. pseudomallei-like strains			
E27	Soil	Thailand	−
E201	Soil	Thailand	−
E205	Soil	Thailand	−
E211	Soil	Thailand	−
E221	Soil	Thailand	−
E229	Soil	Thailand	−
E232	Soil	Thailand	−
E236	Soil	Thailand	−
E217	Soil	Thailand	−
Other *Burkholderia* species			
B. cepacia LMG[c] 1222[T]			−
IMM[d] Xy450			−
ATCC[e] 17478			−
ATCC 17759			−
ATCC 25416			−
B. solanacearum LMG 2299[T]			−
LMG 7005			−
B. pickettii LMG 5942			−
LMG 7005			−
Pseudomonas species			
P. aeruginosa LMG 1242[T]			−
P. alcaligenes LMG 1224 t1			−
P. aureofaciens LMG 1245[T]			−
P. fluorescens bv. 1 LMG 1794[T]			−
bv. 3 LMG 1244			−
bv. 4 LMG 5939			−
P. mendocina LMG 1223			−
P. pseudoalcaligenes LMG 1225[T]			−
P. putida bv. A LMG 2257[T]			−
bv. B LMG 1246 t1			−
LMG 5837			−
LMG 12644			−
P. stutzeri LMG 2243			−
P. diminuta LMG 2089[T]			−
P. vesicularis LMG 2350[T]			−
Other genera			
Comamonas acidovorans LMG 5932			−
Comamonas testosteroni LMG 1787			−
Stenotrophomonas maltophilia			−
ATCC 17448			−
ATCC 17406			−
Klebsiella pneumoniae ATCC 10031			−
NCTC 11228			−
NCTC 10996[f]			−
Klebsiella oxytoca IMM R18[f]			−
Escherichia coli ATCC 11229			−
ATCC 10539			−
ATCC 25922			−
ATCC 29079			−
Acinetobacter baumannii IM R105[f]			−
ATCC 19606			−
Acinetobacter lwoffi IMM R53[f]			−
ATCC 15309			−
Enterobacter cloacae ATCC 23355			−
Citrobacter freundii IMM R24			−
Citrobacter diversus IMM 30			−
Serratia marcescens IMM K10			−
Enterococcus faecium ATCC 19434			−
Streptococcus pneumoniae IMM 46			−
Staphylococcus epidermidis ATCC 12228			−

[a] +, positive; −, negative.
[b] ND, not documented.
[c] LMG, Belgian Collection of Culture Strains, Universiteit Gent, Laboratorium voor Microbiologie, Ghent, Belgium.
[d] IMM, Institute of Medical Microbiology, Hannover, Germany.
[e] ATCC, American Type Culture Collection.
[f] With these strains heat-treated bacterial supernatant had to be used to eliminate nonspecific reactions of bacterial cells with both the specific and control latex reagent.

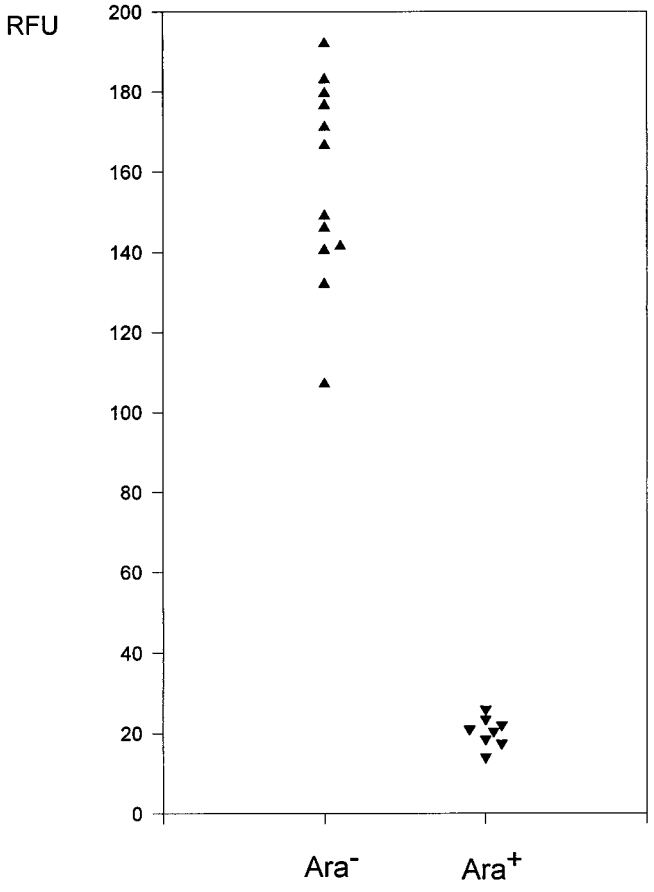

FIG. 1. Reactivity of IgG1 MAb 3015 in an ELISA with 12 *B. pseudomallei* isolates (Ara$^-$, arabinose nonassimilators) and 8 environmental *B. pseudomallei*-like isolates (Ara$^+$, arabinose assimilators). Each point is the mean of duplicate determinations. RFU, relative fluorescence units. RFU values of *B. pseudomallei*-like isolates reacted with MAb 3015 were indistinguishable from the values obtained with unrelated IgG1 and were also indistinguishable from *B. pseudomallei* isolates reacted with unrelated IgG1 (not shown).

min at room temperature. Sensitization of latex particles was performed by incubating equal volumes of particle solutions and antibody solutions (1 mg/ml) for 2 h at 37°C with gentle shaking. The latex particles were then washed three times with distilled water and resuspended in buffer A-BSA to obtain a 5% (wt/vol) suspension.

Latex agglutination assay. The agglutination test was performed by placing 10 μl of the test (MAb 3015 IgG1) or control (unrelated IgG1) latex suspension and 10 μl of buffer A on a black-coated agglutination card. A small portion of a colony was emulsified directly into the drop of buffer and mixed with the latex suspension. Agglutination was detected visually after rotation for 1 min. Agglutination with the control latex was always done in parallel to recognize nonspecific agglutination. When heat-treated bacterial supernatant was used for agglutination, one colony was emulsified in 1 ml of buffer A and heated in a microcentrifuge tube for 5 min at 100°C. The suspension was then centrifuged for 5 min at 13,600 × *g*, and the test was performed with 10 μl of the supernatant.

Results. To test the capability of MAb 3015 specific for a *B. pseudomallei* exopolysaccharide to discriminate between Ara$^+$ *B. pseudomallei*-like strains and Ara$^-$ *B. pseudomallei* strains we first performed an ELISA with heat-treated bacterial cells (Fig. 1). The results indicate that Ara$^+$ *B. pseudomallei*-like organisms, in contrast to Ara$^-$ *B. pseudomallei*, do not synthesize the galactose- and Kdo-containing tetrasaccharide repeating unit recognized by MAb 3015. The results of the latex agglutination test based on MAb 3015 for the identification of *B. pseudomallei* cultures are shown in Table 1. All 74 *B. pseudomallei* strains tested including 12 NCTC strains and 62 clinical and environmental isolates originating from different areas of Southeast Asia, northern Australia, and Africa were positive, showing a strong and rapid agglutination with the MAb 3015 latex, whereas no reaction occurred with the control latex. To further evaluate the specificity of the test a variety of gram-positive and gram-negative bacteria including different *Pseudomonas* and *Burkholderia* ssp. were tested (Table 1). All 56 non-*B. pseudomallei* strains, including the arabinose-positive *B. pseudomallei*-like strains, were negative. A total of 52 strains agglutinated with neither the specific nor the control latex. Four strains showed reactions with both the *B. pseudomallei*-specific latex and the control latex. These nonspecific reactions were absent when bacteria were heat treated and bacterial supernatant was tested. In these tests heat-treated *B. pseudomallei* supernatant served as a positive control and always strongly agglutinated. The sensitized latex particles have now been stable for more than 6 months at 4°C.

Discussion. A previous study described a latex agglutination test for the identification of *B. pseudomallei* isolates from patients in Thailand which was based on a polyclonal rabbit serum raised against *B. pseudomallei* (9). However, this test was also found to be positive with L-arabinose-assimilating *B. pseudomallei*-like strains (12). It seems likely that this cross-reactivity is at least partly due to common lipopolysaccharide epitopes between the avirulent *B. pseudomallei*-like organisms and virulent *B. pseudomallei* (1). Recently, a MAb of the IgM isotype which directly agglutinated *B. pseudomallei* strains from Thailand was described (7). The authors stated that their MAb is probably reactive with an epitope of the lipopolysaccharide. Reactivity with *B. pseudomallei* strains isolated from tropical areas outside of Thailand was not tested. The reactivity of our latex reagent based on the MAb 3015 IgG1, specific for a *B. pseudomallei* exopolysaccharide, with strains from many different locations in Southeast Asia, Australia, and Africa extends our previous finding of a constitutive expression of the *B. pseudomallei* exopolysaccharide independent of the geographical origin of the strain (10). The IgG1 isotype of MAb 3015 used in our test has now proved to be stable for more than 6 months, which is important for routine use in any diagnostic laboratory. In conclusion, this MAb-based test offers a rapid and simple method for the reliable identification of *B. pseudomallei* from different parts of the world. Future studies will determine the applicability of this test for the detection of the exopolysaccharide antigen in the body fluids of patients.

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