Lipopolysaccharides of Pseudomonas spp. that stimulate plant growth: composition and use for strain identification

Weger, L.A. de; Jann, B.; Jann, K.; Lugtenberg, E.J.J.

JOURNAL OF BACTERIOLOGY, Apr. 1987, p. 1441–1446
0021-9193/87/041441-06$02.00/0

Vol. 169, No. 4

Lipopolysaccharides of *Pseudomonas* spp. That Stimulate Plant Growth: Composition and Use for Strain Identification

LETTY A. DE WEGER,[1]* B. JANN,[2] K. JANN,[2] AND BEN LUGTENBERG[1]

Department of Plant Molecular Biology, Botanical Laboratory, 2311VJ Leiden, The Netherlands,[1] and Max-Planck-Institut für Immunobiologie, D-7800 Freiburg-Zähringen, Federal Republic of Germany[2]

Received 14 October 1986/Accepted 31 December 1986

The outer membrane proteins of a series of fluorescent, root-colonizing, plant-growth-stimulating *Pseudomonas* spp. having been characterized (L. A. de Weger et al., J. Bacteriol. 165:585–594, 1986), the lipopolysaccharides (LPSs) of these strains were examined. The chemical composition of the LPSs of the three best-studied plant-growth-stimulating *Pseudomonas* strains WCS358, WCS361, and WCS374 and of *P. aeruginosa* PAO1 as a reference strain was determined and appeared to differ from strain to strain. The 2,6-dideoxy-2-aminosugar quinovosamine was the most abundant compound in the LPS of strain WCS358. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified LPS and of proteinase K-treated cell envelopes revealed ladderlike patterns for most of these strains. These patterns were not substantially influenced by differences in culture conditions. Analysis of proteinase K-treated cell envelopes of 24 root-colonizing *Pseudomonas* spp. revealed a unique band pattern for each strain, suggesting a great variety in the LPS structures present in these root colonizers. Therefore, electrophoretic analysis of LPS can be used for characterization and identification of the fluorescent root-colonizing *Pseudomonas* strains.

In Dutch fields frequently planted with potatoes, yields are reduced by the accumulation of deleterious microorganisms or their products (28). In pot and field experiments it was shown that bacterization of potato tubers with selected root-colonizing, fluorescent *Pseudomonas* spp. diminishes or even abolishes yield reductions (9, 18), presumably in a siderophore-mediated way (5, 23). Efficient colonization of the potato root by the plant-growth-stimulating *Pseudomonas* strain is thought to be very important for yield increase in fields (5). Our selected *Pseudomonas* spp. are efficient root colonizers, as deduced from the fact that they were isolated from the surface of thoroughly washed roots. It is likely that the bacterial surface plays an important role in the interaction between plant and bacterium. For this and other reasons (7), we are interested in the characteristics of the cell surface of these plant-beneficial *Pseudomonas* strains.

In a previous paper we reported our analysis of the membrane proteins of 30 fluorescent root-colonizing *Pseudomonas* spp. by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (7). As judged from their patterns, including the proteins induced by Fe^{3+}-limited growth, most strains were mutually distinguishable. Of these 30 strains, 24 were chosen for use in the present study, which is focused on the lipopolysaccharide (LPS) of these strains.

Research on the bacterial LPS structure in correlation with the interaction of a bacterium with plant tissue has been performed preferentially for interactions of plants with pathogenic bacteria (3, 11, 33, 35). However, a recent publication on the composition of the LPS of saprophytic bacteria (4) might reflect increasing interest in this important group of soil bacteria. Our interest in factors that may be involved in the colonization of the plant root by the plant-growth-promoting *Pseudomonas* spp. prompted us to study in more detail the LPS structure of the three root-colonizing strains WCS358, WCS361, and WCS374. Therefore the compositions of the LPSs of these three strains and of the well-studied *Pseudomonas aeruginosa* strain PAO1 were compared. Furthermore, the LPS of the 24 strains was analyzed by SDS-polyacrylamide gel electrophoresis to study whether the LPS of these *Pseudomonas* strains is a well-preserved structure common to root-colonizing *Pseudomonas* spp. or varies among the different strains. The LPS patterns of all these strains appeared to differ from each other. For this reason analysis of LPS by SDS-polyacrylamide gel electrophoresis can be used for characterization and identification of these root-colonizing *Pseudomonas* strains.

MATERIALS AND METHODS

Strains and growth conditions. The relevant characteristics of the 24 *Pseudomonas* strains used in this study have been published (7). Of the seven strains that are probably identical (7), only strain WCSS358 was used in this study. After diluting stationary-phase cultures 100-fold into fresh culture medium, cells were grown for 64 h under vigorous aeration at 28°C. The following culture media were used. The composition of King B medium, an Fe^{3+}-deficient medium, has been described previously (17). When required, 100 μM $FeCl_3$ was added. Minimal salts medium (30) was supplemented either with 1% glucose as the carbon source or with root exudate from axenically cultivated potato plants. For the isolation of LPS, a stationary-phase culture was diluted 100-fold in fresh King B medium and cultivated for 24 h at 28°C.

Cocultivation of bacteria with sterile potato plantlets. Sterile potato plantlets of the potato cultivar Bintje were obtained from G. S. Bokelman, ITAL Research Institute, Wageningen, The Netherlands. Plantlets were cultivated in culture vessels (type GA7; Magenta Corp., Chicago, Ill.) on medium as described by Murashige and Skooge (24), final pH 5.8, supplemented with 2.0% sucrose and solidified with 0.8% agar. The culture vessels were placed in a growth chamber at 28°C with a day length of 14 h. Prior to cocultivation of plantlets and bacteria, eight sterile plantlets were placed on a metal grid and cultivated on 100 ml of liquid Murashige-Skooge medium. After 1 week the medium was

* Corresponding author.

TABLE 1. Composition of LPS from strains WCS358, WCS361, WCS374, and *P. aeruginosa* PAO1

Strain	Yield (mg/g of cells)	Composition of LPS (% by wt, mean ± SD)[a]											
		KDO[b]	Heptose[b]	P[b]	Glucose[c]	Mannose[c]	Fucose[c]	Rhamnose[c]	Glucosamine[d]	Glucosamine phosphate[d]	Galactosamine[d]	Quinovosamine[d]	Alanine[d]
PAO1	55	2.4 ± 0.2	3.1 ± 0.7	1.3 ± 0.3	5.0 ± 0.8	0	0	3.3 ± 0.1	ND[e]	ND	ND	ND	ND
WCS358	25	3.1 ± 0.3	2.0 ± 0.5	1.3 ± 0.2	9.6 ± 0.8	0	0	0	5.9	1.7	2.0	15–18[f]	0.8
WCS361	50	2.9 ± 0.4	2.1 ± 0.4	1.8 ± 0.3	4.3 ± 0.2	0.6 ± 0.1	0	0.9 ± 0.1	7.8	2.0	2.5	0	1.2
WCS374	5	1.4 ± 0.1	3.2 ± 0.5	0.8 ± 0.1	9.5 ± 0.2	0	1.9 ± 0.2	0.6 ± 0.0	1.7	0.2	0	0	0.7[g]

[a] At least two determinations.
[b] Determined colorimetrically.
[c] Determined by gas-liquid chromatography.
[d] Single determination on an amino acid analyzer.
[e] ND, Not determined.
[f] Estimated from the peak integral.
[g] Besides alanine, glycine (0.8%) was detected in the LPS of strain WCS374 and was predominantly associated with the lipid A fraction.

replaced by bacterial minimal salts medium (30) without any carbon source. A 100-fold-diluted stationary-phase culture of bacteria was cocultivated with the potato plant roots for 3 days at 28°C under gentle rotation. The optical density of the resulting bacterial suspensions varied from 0.6 to 1.0.

Isolation of LPS and cell envelopes. Cells were washed once with PBS (0.9% NaCl buffered with 10 mM sodium phosphate, pH 7.2) and lyophilized. LPS was isolated either after extraction of the cells with hot phenol-water as described by Westphal and Jann (32) or by successive Mg^{2+} and ethanol precipitations after solubilizing the membranes with 2% SDS as described by Darveau and Hancock (6). Contaminating nucleic acids were determined by UV absorbance. Cell envelopes were isolated by differential centrifugation after disruption of the cells by ultrasonic treatment (22).

SDS-polyacrylamide gel electrophoresis. Samples were solubilized by incubation for 15 min at 95°C in the standard sample mixture described previously (22). Solubilized cell envelope samples, containing approximately 1 mg of cell envelope protein per ml, were supplemented with proteinase K (13) to a final concentration of 50 μg/ml and incubated at 56°C for 1 h. Fifteen microliters of the 10-fold-diluted samples was applied per slot. The gel system described previously (22) was used, except that gels contained 13% polyacrylamide instead of 11%. Fast green (22) was used for staining proteinase K-resistant protein fragments, while LPS was stained by the silver-staining procedure described by Tsai and Frasch (29).

Sugar analysis of LPS. To liberate the carbohydrate moiety (core and O-antigenic sidechain) from lipid A, small amounts (1 to 3 mg) of LPS were hydrolyzed in 1 M HCl at 100°C for 15 min. Centrifugation at 10,000 × *g* for 15 min resulted in separation of lipid A (pellet fraction) from the carbohydrate moiety (supernatant fraction).

For analysis of neutral sugars by gas-liquid chromatography, LPS was hydrolyzed in 2 N trifluoroacetic acid by incubation for 1 h at 120°C. The sugars were converted to their alditol acetate derivatives (1) and analyzed by gas-liquid chromatography at 180°C with a gas chromatograph (Becker model 420) with a glass column packed with 3% ECNSS-M on Chromosorb Q (Applied Science Laboratories) and equipped with an integrator (Shimadzu C-R1B).

For thin-layer chromatography, LPS was hydrolyzed in 1 M HCl (neutral sugars) or 6 M HCl (aminosugars) at 100°C for 4 h. The hydrolysates were lyophilized and dissolved in demineralized water. Approximately 20 μg of hydrolyzed LPS was spotted on Kieselguhr Silica Gel G plates (Merck, Darmstadt). For resolving amino compounds, chromatograms were developed in solvent system 1 (pyridine-ethyl acetate-acetic acid-water, 35:35:7:21 by vol) and stained with ninhydrin (31). For resolving neutral sugars, solvent system 1 or 2 (acetone-chloroform-water, 85:10:5 by vol) was used to develop the chromatograms, and spots were detected by using an aniline-phthalate spray (31).

For identification of the most abundant aminosugar in the LPS of strain WCS358, the hydrolyzed LPS was analyzed by paper electrophoresis on 2043 paper (Schleicher & Schuell) in pyridine-acetic acid-water (10:4:86, by vol), pH 5.4, at 40 V/cm. The aminosugar was identified by using the Elson-Morgan reagent (26) and after periodate treatment by using the Edwards and Waldron reagent (8).

Amino acids and aminosugars were quantitatively analyzed after hydrolysis in 4 M HCl for 18 h and subjected to amino acid analysis in a Chromakon 500 amino acid analyzer. Since quinovosamine was not available as a pure sugar, the value for the amount of quinovosamine was estimated from the peak integral.

Other analytical procedures. Heptose was determined as described by Wright and Reber (34), with manno-heptulose as the reference. 2-Keto-3-deoxyoctanate (KDO) was measured by the thiobarbituric acid method (16), with commercial KDO (Sigma Chemical Co., St. Louis, Mo.) as a standard. Phosphate was assayed as described by Ames and Dubin (2).

RESULTS

Isolation of LPS. LPS of strains WCS358, WCS361, WCS374, and *P. aeruginosa* PAO1 was isolated by the hot phenol-water method (32) and by the method described by Darveau and Hancock (6). For the strains under study the latter method proved to be superior in both yield and purity. This procedure yielded 5 to 55 mg of LPS per g of cells (dry weight), depending on the strain (Table 1), with strain WCS374 always giving the lowest yield. Contaminating nucleic acids never exceeded 1%. In addition, no protein could be detected in LPS preparations of strains WCS358, WCS361, and WCS374 when examined by SDS-polyacrylamide gel electrophoresis followed by fast green staining. Since this staining method reveals protein bands of 0.5 μg or more, we concluded that the percentage of contaminating polypeptide was less than 0.5% by weight. In the LPS preparation of strain PAO1 a vague elongated band consisting of a (proteinase K-resistant) polypeptide fragment(s) was detectable in fast-green-stained gels, corresponding to an estimated polypeptide contamination of 1 to 2%.

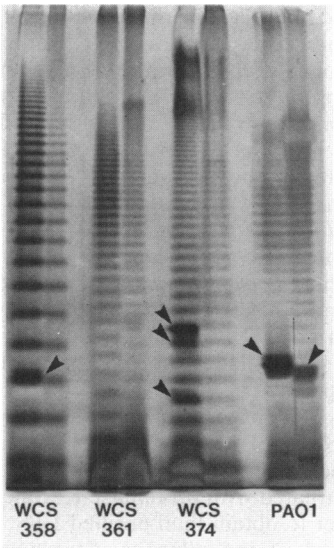

FIG. 1. Band patterns of silver-stained preparations of proteinase K-treated cell envelopes (left lanes) and purified LPS (right lanes) after analysis by SDS-polyacrylamide gel electrophoresis. Arrows indicate bands that can also be visualized with fast green staining and which therefore presumably represent protein fragments resistant to proteinase K. For strain WCS361 the middle and lower part of the LPS profile is not identical to the profile of proteinase K digests. Similar differences in this part of the profile were observed among different proteinase K digests of cell envelopes of this strain (compare the left lane for strain WCS361 of this figure with lane 18 in Fig. 3).

LPS preparations stained after SDS-polyacrylamide gel electrophoresis showed different ladderlike patterns for each strain (Fig. 1). Cell envelopes treated with proteinase K revealed the same patterns as purified LPS, except for some extra bands (indicated by arrows in Fig. 1). These extra bands were also observed in fast-green-stained gels, indicating that these bands are proteinase K-resistant polypeptide fragments. Proteinase K-resistant bands were also observed in proteinase K digests of *Coxiella burnetii* cells (12).

Composition of LPS. Colorimetric determinations showed the presence of various amounts of KDO, heptose, and phosphate in the LPS of strains WCS358, WCS361, WCS374, and *P. aeruginosa* PAO1 (Table 1). Analysis of the LPS by gas-liquid chromatography revealed differences in neutral sugar composition among the various strains (Table 1). Our results confirmed previous ones (19) which indicated that the LPS of *P. aeruginosa* contains glucose and rhamnose. Glucose was present in all three plant-root-colonizing *Pseudomonas* strains (Table 1). Besides glucose, no neutral sugars were detected in strain WCS358, while in strain WCS361 low levels of mannose and rhamnose were detected (Table 1). In strain WCS374 glucose as well as the two 6-deoxysugars, rhamnose and fucose, were present (Table 1).

Analysis of the amino compounds indicated the presence of alanine, glucosamine, and its phosphorylated derivative in the LPS of all three root-colonizing strains and of galactosamine in strains WCS358 and WCS361 (Table 1). Furthermore, the LPS of WCS358 contained another aminosugar as the most abundant constituent. In paper electrophoresis this aminosugar had a mobility relative to glucosamine (M_{GlcN}) of 1.06. It could be stained with the Elson-Morgan reagent, which is indicative of a 2-deoxy-2-aminosugar, and after periodate treatment with the Edwards and Waldron reagent, which is indicative of a 6-deoxy group. Thus, the aminosugar was probably a 2,6-dideoxy-2-amino sugar. It eluted from the amino acid analyzer with an elution time relative to glucosamine (t_{GlcN}) of 1.126. This was identical with the elution time of 2,6-dideoxy-2-amino-glucose (quinovosamine; t_{GlcN}, 1.123) and different from those of rhamnosamine (t_{GlcN}, 1.088) and fucosamine (t_{GlcN}, 1.178). These results indicate that the LPS from WCS358 contains, in addition to glucosamine, 2,6-dideoxy-2-amino-glucose (quinovosamine).

The presence of glucosamine phosphate in hydrolysates of LPS indicates incomplete hydrolysis of this constituent, which is characteristic of lipid A. To assess the distribution of the aminosugars between lipid A and the carbohydrate moiety (core and O-antigenic side chain), both of these fractions were analyzed for aminosugars. All lipid A fractions contained glucosamine and glucosamine phosphate and practically no other aminosugars. The carbohydrate fraction from strain WCS374 contained only glucosamine, that from strain WCS361 contained glucosamine and galactosamine in the molar ratio of 1:1, and that from strain WCS358 contained galactosamine and quinovosamine in the molar ratio of 1:10. Alanine was predominantly found in the carbohydrate moiety of these strains.

Influence of culture conditions on the LPS patterns in silver-stained gels. Cell envelopes of strains WCS358, WCS361, and WCS374 were treated with proteinase K. Growth in minimal medium with either glucose or root exudate from sterile potato plants as the carbon source resulted in LPS patterns indistinguishable from those observed after growth in King B medium. Neither addition of 100 μM $FeCl_3$ to the medium nor variation in growth temperature from 4 to 44°C altered the LPS ladder patterns significantly (data not shown). Also, the growth phase at 28°C had no effect on the LPS patterns of strains WCS361 and WCS374. An extension of the ladder pattern in the high-molecular-weight region of the gel was observed for strain WCS358 when cells arrived at the stationary phase (Fig. 2).

LPS patterns of a collection of antagonistic *Pseudomonas* root isolates. Cell envelopes of 24 *Pseudomonas* root isolates (7) were treated with proteinase K, and the LPS species were electrophoretically separated and stained with the silver reagent. The majority of the root isolates showed ladderlike LPS patterns (Fig. 3). Instead of discrete bands, elongated spots were observed in the LPS patterns of strains WCS312, WCS317, WCS324, and E6. Only one strain, WCS429, showed a pattern consistent with LPS lacking the O-antigenic side chain.

Each of these 24 strains showed a unique LPS pattern, except for strains WCS374 and WCS375, which are colony variants (7). Strain WCS358 and the six strains (WCS345, WCS348, WCS357, WCS359, WCS360, WCS364) mentioned in a previous paper (7) as most likely being descendants of one ancestor had indistinguishable LPS patterns.

DISCUSSION

Composition of LPS. Our results on the composition of the LPS of *P. aeruginosa* PAO1 (Table 1) were very similar to those reported by Kropinski et al. (19), except for the phosphorus content, which was lower in our assays. The relative amounts of KDO and heptose (Table 1) were very similar in the three root-colonizing *Pseudomonas* strains and *P. aeruginosa* PAO1 (2.5 to 3% and 2 to 3%, respectively),

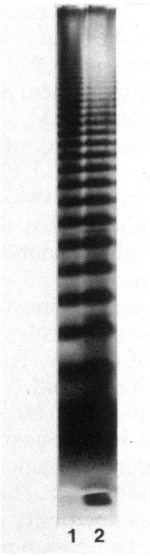

FIG. 2. Silver-stained LPS patterns of cell envelopes of strain WCS358 treated with proteinase K after growth for 64 h (lane 1) or 8 h (lane 2) in King B medium. The additional weak band in the middle of the ladder pattern in lane 2 was not observed in other proteinase K digests of these cell envelopes.

except that the KDO content in strain WCS374 was slightly lower (1.4%). Glucose was present as the major neutral sugar in each of these three strains. Low levels of mannose and rhamnose were detected in the LPS of strain WCS361, whereas fucose and rhamnose were found in the LPS of strain WCS374 (Table 1). The LPS of strain WCS358 did not contain other neutral sugars.

Analysis of the amino compounds revealed that the lipid A from strains WCS358, WCS361, and WCS374 contained glucosamine and its phosphorylated derivative but no other aminosugars. The aminosugar composition of the carbohydrate moiety (core and O-antigenic side chain) differed among the strains (Table 1). In strain WCS358, the very low relative content of galactosamine makes it difficult to envisage both galactosamine and quinovosamine as part of the repeating unit. Since the carbohydrate fraction consists of O-antigenic side chain linked to the core, the possibility exists that in contrast to quinovosamine, galactosamine is not a constituent of the O-antigenic side chain but of the core of the LPS of strain WCS358.

Quinovosamine has been reported to be present in the LPS of many other bacterial species (e.g., some *P. aeruginosa* strains [20], *Salmonella* spp., *Proteus vulgaris* [21], *Vibrio cholerae* [14], and *Rhizobium leguminosarum* [27]). In the LPS of *P. aeruginosa* PAO, another 2,6-dideoxy-2-aminohexose, fucosamine (2,6-dideoxy-2-aminogalactose), was found (17). Like the quinovosamine in strain WCS358, the 2,6-dideoxy-2-aminosugars in the LPS of several *P. aeruginosa* strains were shown to be constituents of the O-antigenic side chain (19, 20).

Anderson (4) detected both rhamnose and glucose in both pathogenic (*Pseudomonas syringae* strains) and saprophytic *Pseudomonas* spp. (*P. fluorescens* and *P. aeruginosa*). Saprophytic *P. putida* strains, which are distinguished from other *Pseudomonas* spp. by their agglutination by a plant arabinogalactan protein complex (15), showed a unique LPS composition as (i) they contained glucose as the major neutral sugar, (ii) they had a high ratio of amino over neutral sugars, and (iii) they lacked rhamnose and fucose (4). We found a similar result for strain WCS358. However, the features mentioned do not seem to be a general property of the LPS of saprophytic *P. putida* strains, since the LPS of the other *P. putida* strain in our study, WCS361, neither contained high levels of aminosugars nor lacked rhamnose. Fucose, reported to be present in *P. fluorescens* and *P. syringae* strains (4), was also found in the plant-growth-promoting *P. fluorescens* strain WCS374.

In conclusion, the composition of the LPS of the plant-growth-promoting *Pseudomonas* strains is comparable to that of other gram-negative bacteria. No common features were found in their LPSs, suggesting that the LPSs of *Pseudomonas* spp. isolated from the roots of potato plants do not share specific characteristics.

LPS patterns of plant-growth-promoting *Pseudomonas* spp. Analysis by SDS-polyacrylamide gel electrophoresis revealed the same ladderlike patterns for purified LPS and for cell envelopes treated with proteinase K. Since the latter are faster and easier to obtain than purified LPS, we used these preparations to study the influence of various growth conditions on the LPS patterns and to study the LPS patterns of 24 fluorescent root-colonizing *Pseudomonas* strains. No influence of varying the growth conditions was observed, except that for strain WCS358 a slight increase in the ladder pattern in the high-molecular-weight part of the gel was detected when the cells entered the stationary phase (Fig. 2). Apparently a slight change in the size distribution of the LPS molecules in favor of LPS molecules with increasing length of the O-antigenic side chains took place. Analysis of cell envelopes treated with proteinase K resulted for most of the 24 strains in ladderlike patterns (Fig. 3). This multitude of bands observed in the LPS patterns is supposed to be due to LPS molecules having varying lengths of O-antigenic side chains (10, 25). For each strain a different pattern was observed, except WCS374 and WCS375 (Fig. 3), which are colony variants (7). This result showed that the LPSs of

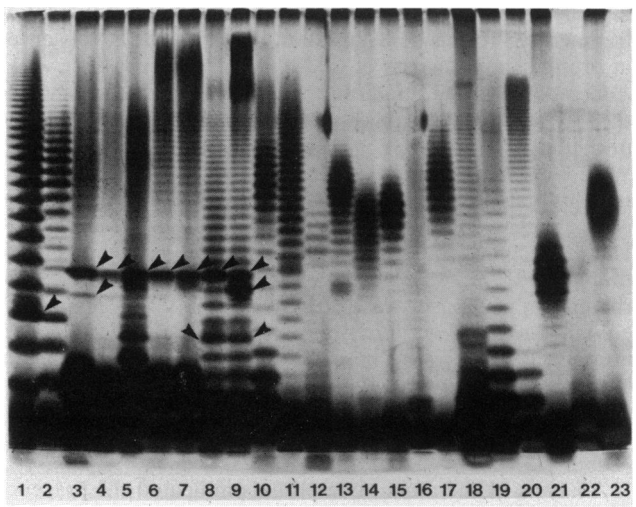

FIG. 3. Silver-stained patterns of proteinase K-treated cell envelopes obtained after SDS-polyacrylamide gel electrophoresis. Lanes: 1, WCS358; 2, A1; 3, WCS141; 4, WCS312; 5, WCS317; 6, WCS321; 7, WCS327; 8, WCS374; 9, WCS375; 10, WCS007; 11, WCS085; 12, WCS134; 13, WCS307; 14, WCS314; 15, WCS315; 16, WCS324; 17, WCS326; 18, WCS361; 19, WCS365; 20, WCS366; 21, WCS379; 22, WCS429; 23, E6. Arrows indicate protein fragments resistant to proteinase K. For details see the legend to Fig. 1.

root-colonizing *Pseudomonas* strains are not well-preserved structures.

Previously we reported the analysis of the membrane proteins of the *Pseudomonas* strains used in this study (7). Most of these strains were mutually distinguishable by their membrane protein pattern. However, a few strains were hard to discriminate by their protein patterns (e.g., the pairs WCS358 and A1 and WCS141 and WCS312 [7]). Analysis of the LPSs of these strains revealed that they were actually distinct. Since we showed that the LPS patterns of the *Pseudomonas* strains tested are not substantially influenced by culture conditions and that the ladder patterns are unique for each of these strains, they can be used to identify each individual strain. Therefore LPS patterns, in combination with the membrane protein patterns (7), provide a powerful tool to accurately identify these fluorescent root-colonizing *Pseudomonas* spp., e.g., reisolates from field experiments.

ACKNOWLEDGMENTS

We thank Lia van der Vlugt and Harold Klaassen for technical assistance.

These investigations were supported by the Netherlands Technology Foundation (STW).

LITERATURE CITED

1. **Albersheim, P., D. J. Nervins, and A. Kaar.** 1967. A method for the analysis of sugar in plant cell wall polysaccharides by gas-liquid chromatography. Carbohydr. Res. **5**:340–345.
2. **Ames, B. N., and D. T. Dubin.** 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. **235**:769–775.
3. **Anderson, A. J.** 1980. Unique aspects of cell surface polysaccharides of *Pseudomonas phaseolicola* as demonstrated by bacteriophage specificity. Can. J. Microbiol. **26**:1422–1427.
4. **Anderson, A. J.** 1984. Differences between lipopolysaccharide compositions of plant-pathogenic and saprophytic *Pseudomonas* spp. Appl. Environ. Microbiol. **48**:31–35.
5. **Bakker, P. A. H. M., J. G. Lamers, A. W. Bakker, J. D. Marugg, P. J. Weisbeek, and B. Schippers.** 1986. The role of siderophores in potato tuber yield increase by *Pseudomonas putida* in a short rotation of potato. Neth. J. Plant. Pathol. **92**:249–256.
6. **Darveau, R. P., and R. E. W. Hancock.** 1983. Procedure for isolation of bacterial lipopolysaccharides from both smooth and rough *Pseudomonas aeruginosa* and *Salmonella typhimurium* strains. J. Bacteriol. **155**:831–838.
7. **de Weger, L. A., R. van Boxtel, B. van der Burg, R. Gruters, F. P. Geels, B. Schippers, and B. Lugtenberg.** 1986. Siderphores and outer membrane proteins of antagonistic plant-growth-stimulating, root-colonizing *Pseudomonas* spp. J. Bacteriol. **165**:585–594.
8. **Edwards, J. T., and D. M. Waldron.** 1952. The detection of deoxy-sugars, glycals, and methyl pentoses in paper partition chromatography. J. Chem. Soc., p. 3631–3634.
9. **Geels, F. P., and B. Schippers.** 1983. Reduction of yield in high frequency potato cropping soil after seed tuber treatments with antagonistic fluorescent *Pseudomonas* spp. Phytopathol. Z. **108**:207–214.
10. **Goldman, R. C., and L. Leive.** 1980. Heterogeneity in antigenic side chain length in lipopolysaccharide from *Escherichia coli* O111 and *Salmonella typhimurium* LT2. Eur. J. Biochem. **107**:145–153.
11. **Graham, T. L., L. Sequeira, and T.-S. R. Huang.** 1977. Bacterial lipopolysaccharides as inducers of disease resistance in tobacco. Appl. Environ. Microbiol. **34**:424–432.
12. **Hackstadt, T., M. G. Peacock, P. J. Hitchcock, and R. L. Cole.** 1985. Lipopolysaccharide variation in *Coxiella burnetii*: intrastrain heterogeneity in structure and antigenicity. Infect. Immun. **48**:359–365.
13. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
14. **Jann, B., K. Jann, and G. O. Beyaert.** 1973. 2-Amino-2,6-dideoxy-D-glucose (D-quinovosamine): a constituent of the lipopolysaccharides of *Vibrio cholerae*. Eur. J. Biochem. **37**: 531–534.
15. **Jasalavich, C. A., and A. J. Anderson.** 1981. Isolation from legume tissues of an agglutinin of saprophytic pseudomonads. Can. J. Bot. **59**:264–271.
16. **Keleti, G., and W. H. Lederer.** 1974. Handbook of micromethods for the biological sciences, p. 74–75. Van Nostrand Reinhold Co., Inc., New York.
17. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. **44**:301–307.
18. **Kloepper, J., J. Leong, M. Teintze, and M. N. Schroth.** 1980. Enhanced plant growth by siderophores produced by plant-growth-promoting rhizobacteria. Nature (London) **286**:885–886.
19. **Kropinski, A. M., L. C. Chan, and F. H. Milazzo.** 1978. The extraction and analysis of lipopolysaccharides from *Pseudomonas aeruginosa* strain PAO and three rough mutants. Can. J. Microbiol. **25**:390–398.
20. **Kropinski, A. M., J. Kuzio, B. L. Angus, and R. E. W. Hancock.** 1982. Chemical and chromatographic analysis of lipopolysaccharide from an antibiotic-supersusceptible mutant of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **21**:310–319.
21. **Lüderitz, O., J. Gmeiner, B. Kickhöfen, H. Mayer, O. Westphal, and R. W. Wheat.** 1968. Identification of D-mannose and quinovosamine in *Salmonella* and related bacteria. J. Bacteriol. **95**:490–494.
22. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the "major outer protein" of *Escherichia coli* K12 into four bands. FEBS Lett. **58**:254–258.
23. **Marugg, J. D., M. van Spanje, W. P. M. Hoekstra, B. Schippers, and P. J. Weisbeek.** 1985. Isolation and analysis of genes involved in siderophore biosynthesis in plant-growth-stimulating *Pseudomonas putida* WCS358. J. Bacteriol. **164**:563–570.
24. **Murasige, T., and F. Skooge.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**:473–497.
25. **Palva, E. T., and P. H. Mäkelä.** 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Eur. J. Biochem. **107**:137–143.
26. **Partridge, S. M.** 1948. Filter-paper partition chromatography of sugars. Biochem. J. **42**:238–248.
27. **Planqué, K., J. J. van Nierop, A. Burgers, and S. G. Wilkinson.** 1979. The lipopolysaccharide of free-living and bacteroid forms of *Rhizobium leguminosarum*. J. Gen. Microbiol. **110**:151–159.
28. **Schippers, B., F. P. Geels, O. Hoekstra, J. G. Lamers, C. A. A. A. Maenhout, and K. Scholte.** 1985. Yield depressions in narrow rotations caused by unknown microbial factors and their suppression by selected Pseudomonads, p. 127–130. *In* C. A. Parker, K. J. Moore, P. T. W. Wong, A. D. Rovira, and J. F. Kollmorgen (ed.), Ecology and management of soil-borne plant pathogens. The American Phytopathology Society, St. Paul, Minn.
29. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
30. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
31. **Waldi, D.** 1965. Spray reagent for thin layer chromatography, p. 483–502. *In* E. Stahl (ed.), Thin layer chromatography: a laboratory handbook. Springer-Verlag, Berlin.
32. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides: extraction with phenol-water and further applications of the procedure, p. 83–91. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry, vol. 5. Academic Press, Inc., New York.

33. **Whatley, M. H., J. S. Bodwin, B. Lippincott, and J. A. Lippincott.** 1976. Role for *Agrobacterium* cell envelope lipopolysaccharide in infection site attachment. Infect. Immun. **13**:1080–1083.
34. **Wright, B. G., and P. A. Rebers.** 1972. Procedure for determining heptose and hexose in lipopolysaccharides—a modification of the cysteine-sulfuric acid method. Anal. Biochem. **49**:307–319.
35. **Zamze, S. E., A. R. W. Smith, and R. C. Hignett.** 1985. Composition of lipopolysaccharide from *Pseudomonas syringae* pv. *morsprunorum* of differing host specificity and virulence. J. Gen. Microbiol. **131**:1941–1950.