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Diversity of antifungal and plant-associated *Serratia plymuthica* strains

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G. BERG. 2000. A total of 21 plant-associated *Serratia plymuthica* strains were characterized phenotypically by their nutritional patterns, susceptibility to antibiotics, antifungal and haemolytic properties, and genotypically by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA, PCR fingerprints using BOX primers (BOX-PCR) and pulsed-field gel electrophoresis (PFGE) after digestion with *Spe*I.
All of the investigated strains demonstrated antifungal activity *in vitro* against fungal pathogens while only six strains produced the antifungal antibiotic prodigiosin. Haemolytic activity and antibiotic resistance patterns were investigated to assess the risk associated with the use of isolates in plant protection. The strains were haemolytic at human-relevant temperatures. The level of resistance to antibiotics was low. This work shows that BOX-PCR and PFGE are useful fingerprinting methods to characterize *Ser. plymuthica* strains, although the discriminatory effect between the two methods differed. Computer-assisted analysis of phenotypic and genotypic features demonstrated relationships between the origin of isolation, the production of prodigiosin and the molecular fingerprint.

INTRODUCTION

The Gram-negative bacterium *Serratia plymuthica* has frequently been associated with plants and has been isolated from the rhizosphere of wheat, oat, cucumber, maize, oilseed rape and potato (Alström and Gerhardson 1988; Grimmont and Grimmont 1992; Kalbe *et al.* 1996). Investigations have indicated a potential role for this species in biotechnology and *Ser. plymuthica* has been used as a biological control agent of fungal plant pathogens in agriculture (Kloepper *et al.* 1992; Stanley *et al.* 1994; McInroy and Kloepper 1995; McCullagh *et al.* 1996; Berg *et al.* 1998; Frankowski *et al.* 1998). Rhizobacteria which establish positive interactions with plant roots such as *Ser. plymuthica*, play a key role in agricultural environments and are promising candidates for use in sustainable agriculture (Di Cello *et al.* 1997; Whipps 1997). Furthermore, *Ser. plymuthica* strains have been reported to be contaminants in the fish industry (Lopez-Sabater *et al.* 1996; Lyhs *et al.* 1998) and in contact lens cases (Midelfart *et al.* 1996). Other species of the genus *Serratia* such as *Ser. marcescens* are known nosocomial pathogens (Grimmont and Grimmont 1992). Although, *Ser. plymuthica* is not generally recognized as an organism capable of causing serious human infections, it is becoming a significant pathogen (Clark and Janda 1985; Horowitz *et al.* 1987; Zbinden and Blass 1988; Reina *et al.* 1992; Domingo *et al.* 1994; Carrero *et al.* 1995).

Typing methods of bacteria involve characterization below the species level to enable subdivision into subgroups or types. Molecular typing methods for this particular bacterial species have not been evaluated. However, typing methods exist for the related species *Ser. marcescens* in form of restriction fragment length polymorphism by pulsed-field gel electrophoresis (PFGE) (Miranda *et al.* 1996; Traub *et al.* 1996; Chetoui *et al.* 1998), random amplified polymorphic DNA (RAPD) analysis by arbitrarily primed PCR (Hejazi *et al.* 1997) and ribotyping (Chetoui *et al.* 1998).

In this study, 21 isolates of plant-associated *Ser. plymuthica* including the type strain were investigated by genotypic and phenotypic fingerprinting methods to characterize the intraspecific diversity of this species and to find relationships between them. Molecular methods (denaturing gradient gel electrophoresis [DGGE] of PCR-amplified 16S rDNA) and fingerprinting techniques (BOX-PCR, PFGE) were applied and evaluated. Antibiotic resistance patterns and haemolytic activity were included in the study

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of phenotypic characteristics to provide some indication of the pathogenicity of *Ser. plymuthica* towards humans. The study is important not only for understanding the biodiversity of the species, but also for epidemiological and ecological investigations in agriculture and in human medicine and for patenting bacterial strains for biotechnological applications.

MATERIALS AND METHODS

Bacterial strains

A total of 21 isolates were included in the study (Table 1). *Ser. plymuthica* DSM 4540 (= ATCC 183, type strain) was used as a reference strain for comparison.

Identification and metabolic fingerprint

All isolates were identified using BIOLOG system (BIOLOG Inc., Haward, CA, USA). To obtain the metabolic fingerprints by BIOLOG, strains were cultivated on tryptic soy agar (TSA) (Gibco, Eggenstein, Germany) for 24 h at 30 °C. Bacterial cells were harvested and suspended in 0·85% NaCl solution. One hundred and fifty microlitres of the suspension with an O.D. of 0·2 (3×10^8 cells ml^{-1}) was transferred into BIOLOG GN (system für Gram-negative aerobic bacteria) microplates with a multichannel pipette. Results were read visually after 24 h incubation at 30 °C and analysed in the statistical databank (MicroLog™ system, Biolog Inc.). Strains were tested in duplicate to determine reproducibility of the technique. In addition, strains were identified using API system (API20E, BioMérieux, Mercy Etoile, France) (Kalbe *et al.* 1996).

Bioassay for *in vitro* inhibition of fungal growth

Antifungal activity was determined by a paired *in vitro* assay on Waksman agar containing 5 g proteose-peptone (Merck, Darmstadt, Germany), 10 g glucose (Merck), 3 g meat extract (Chemex, Munich, Germany), 5 g NaCl (Merck), 20 g agar (Difco, Detroit, MI, USA), 1 l distilled water, pH 6·8. A suspension of hyphal fragments of fungi was plated on agar and bacteria were streaked as a broad band. Zones of inhibition were measured after 5 d of incu-

Table 1 Type and reference strains of *Serratia plymuthica* included in this study

No	Code	Isolation dates*	Source or reference
s1	DSM 8571†	Rhizosphere of oilseed rape; Rostock 1993	Kalbe *et al.* (1996)
s2	DSM 8572†	Rhizosphere of oilseed rape; Rostock 1993	Kalbe *et al.* (1996)
s3	C2	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s4	C3	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s5	C38	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s6	C44	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s7	C45	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s8	C46	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s9	C47	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s10	DSM 12502†	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s11	C50	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s12	C52	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s13	C53	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s14	C54	Rhizosphere of oilseed rape; Pölchow 1994	Kalbe *et al.* (1996)
s15	P485	Rhizosphere of oilseed rape; Biestow 1995	Kalbe *et al.* (1996)
s16	S1	Rhizosphere of strawberry; Goorstorf,1998	Culture Collection‡
s17	JE1	Caulsophere of potato; Groß Lüsewitz 1998	Culture Collection‡
s18	L-12–6-12	Rhizosphere of potato; Groß Lüsewitz 1997	Lottmann *et al.* (1999)
s19	L19–6-1	Rhizosphere of potato; Groß Lüsewitz 1997	Lottmann *et al.* (1999)
s20	L27–6-4	Rhizosphere of potato; Groß Lüsewitz 1997	Lottmann *et al.* (1999)
s21	DSM 4540†	Type strain	Grimmont *et al.* (1977)

*Microhabitat (origin), locality (town in Mecklenburg-Pomerania) and date (year) of the sample.
†DSM – Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).
‡Culture collection of the Microbiology department at the University of Rostock/Germany.

bation at 20 °C and evaluated according to Berg (1996). The fungal strains were obtained from Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany): *Verticillium dahliae* DSM 12216 and *Rhizoctonia solani* DSM 63010.

Haemolytic activity

Clearing zones were observed on blood agar (Gibco) containing 5% horse blood (BioMérieux) after incubation at 30 and 37 °C, respectively, for 24 h.

Production of prodigiosin

Bacteria were cultivated on peptone glycerol agar containing 5 g bacto peptone (Difco), 10 ml glycerol, 20 g bacto-agar (Difco) and 1 l distilled water at 30 °C for 48 h. Cells were extracted from Petri dishes were harvested and dissolved in 25 ml acetone for 30 min. The solution was centrifuged at 10 000 *g* for 10 min. The supernatant was then purified by gel filtration on Sephadex LH20 (Pharmacia, Uppsala, Sweden) using methanol-distilled water (1 : 1 v/v) as eluting solvent. The active fractions were concentrated under vacuum (50 °C) and then the dry residue was dissolved in 1 ml of ethanol. The characteristic spectrum of the red pigment was proved according to Süssmuth *et al.* (1987).

Antibiotic resistance pattern

The antibiotic resistance test to determine the susceptibility of bacteria to relevant antibiotics in a semisolid medium similar to the reference method (agar dilution method) was carried out with ATB Antibiogram ATB Pseud (BioMérieux) according to the manufacturer's recommendations. The following concentrations of antibiotics were used (mg l^{-1}): ampicillin – 2 and 8, mezlocillin – 4 and 16, ampicillin-sulbactam – 2/8 and 8/8, Piperacillin-tazobactam – 4/4 and 32/4, cefazolin – 4, cefixim – 1 and 4, cefuroxim – 4 and 8, cefotaxim – 2 and 8, ceftacidim – 1 : 1, imipenem – 4, gentamicin – 1 and 4, tobramycin – 1 and 4, ofloxacin – 1, tetracyclin – 1 and 4, cotrimoxazol – 16 and 64, trimethoprim – 4, nitroxolin – 8, nitrofurantoin – 64 and 256. The results obtained classified the strain as susceptible (no growth or resultant turbidity at both concentrations), intermediate (growth and turbidity only at the lowest concentration), or resistant (growth or turbidity at both concentrations) to each antibiotic.

Molecular typing by DGGE

Total DNA was extracted from the bacterial cell pellet (Wilson 1987). Amplification of the bacterial 16S rDNA fragment between positions 968–1401 (F968; R1401 of the *Escherichia coli* numbering) by PCR, and separation of the products by DGGE was done as described previously (Heuer *et al.* 1997). Approximately equal amounts of PCR-products (1–2 μl) were applied to DGGE, as judged from an ethidium bromide stained agarose gel. A denaturing gradient of 3·2–4·6 mol l^{-1} urea plus 16·0–23·2% (v/v) formamide was used for DGGE (*D*code system, BioRad, Munich, Germany), and gels were run in TAE buffer (40 mmol l^{-1} Tris-acetate, 1 mmol l^{-1} EDTA, pH 8·0) at 60 °C and a constant voltage of 180 V for 4 h. Acid silver staining was used for detection of DNA in DGGE-gels (Riesner *et al.* 1989).

Molecular typing by BOX-PCR

Genomic DNA from each strain was extracted by the method of Wilson (1987). BOX element oligonucleotide primers with the sequence of 5′-CTACGGCAAGG CGACGCTGACG-3′ were synthesized by MWG Biotech (Ebersberg, Germany). PCR was performed as previously described by Rademaker and De Bruijn (1997). To ensure reproducibility, PCRs were carried out with DNA from duplicate colonies and in duplicate with DNA from one of the preparations.

Molecular typing by pulsed field gel electrophoresis (PFGE)

Strains were grown overnight in 10 ml volumes of Luria broth (Difco). After centrifugation at 13 000 rev min^{-1} for 1 min, each cell pellet was washed twice and resuspended in 1 ml of saline EDTA (SE) buffer (25 mmol l^{-1} EDTA, pH 7·4; 75 mmol l^{-1} NaCl). Agarose plugs were made from a 1 : 1 mixture of 1·6% low-melting-point agarose (Biometra, Göttingen, Germany) and the cell suspension (O.D. = 0·6–0·8). Each plug was placed in 5 ml of lysis buffer (10 mmol l^{-1} Tris-HCl [pH 7·6] 100 mmol l^{-1} EDTA [pH 8·0] 50 mmol l^{-1} NaCl, 0·2% deoxycholic acid, 1% *N*-lauroyl sarcosine, 2 mg of lysozyme) for 3 h at 35 °C with shaking. Samples were then treated for 16 h at 50 °C with the same volume of NDS buffer (0·5 mol l^{-1} EDTA, and 1% *N*-lauroyl sarcosine [pH 8·0]) containing 2 mg ml^{-1} of proteinase K. After three 2 h washes with TE buffer (10 mmol l^{-1} Tris-HCl, 0·1 mmol l^{-1} EDTA [pH 8·0]), the agarose plugs were stored in TE buffer at 4 °C for subsequent PFGE. For restriction endonuclease digestion, 2 mm plug slices were equilibrated in 300 μl of the appropriate 1 $\times$ restriction endonuclease buffer for 3 h at 37 °C, to which 25 U of *Spe*I (New England Biolabs, Schalbach, Germany) was added. The reaction mixture was incubated at the manufacturer-suggested incubation temperature. DNA fragments were separated on a 1% PFGE agarose gel

(peqLab, Erlangen, Germany) in $0{\cdot}5 \times$ TBE buffer (45 mmol l^{-1} Tris-borate, 1 mmol l^{-1} EDTA [pH 8·0]) at 10 °C using a contour-clamped homogeneous electrical field (Rotaphor V system, Biometra). With the voltage set at 200 V cm^{-1}, pulse times ranged from 5 to 60 s over 28 h with linear ramping. The procedure was repeated at least twice for each isolate to determine the reproducibility of the results. DNA bands were visualized by ethidium bromide staining.

Statistical analysis

Data from the physiological characterization study were converted to a binary code and interisolate relationships were measured by the Euclidian metric unweighted pair-group average (UPGMA) method by using the program STATISTICA (StatSoft, Hamburg, Germany). Molecular fingerprint patterns generated by each strain were compared and grouped by using the GelCompare program (Kortrijk, Belgium).

RESULTS

A total of 21 *Serratia plymuthica* isolates from different environmental sources listed in Table 1 were compared by their nutritional properties using the standardized BIOLOG system. The oxidative utilization of 95 different carbon sources was tested for each isolate using BIOLOG GN plates. While the isolates exhibited heterogeneity in their carbon utilization profiles, the BIOLOG system identified all strains precisely as *Ser. plymuthica* with high identification rates (ID) ranging from 0·664 to 0·962 (Table 2). In the BIOLOG system, the similarity index must be at least 0·5 to be considered acceptable. Similarity between isolates was analysed statistically using a cluster analysis. On the basis of 75% similarity it was possible to arrange the isolates into three groups (Table 3).

The isolates were screened for their ability to suppress the fungal plant pathogens *Verticillium dahliae* and *Rhizoctonia solani* in an *in vitro* bioassay. All isolates were antifungal while the efficiency was strain specific. In general, the antifungal activity of *Ser. plymuthica* isolates against *Rhizoctonia solani* was lower. All of the plant-asso-

Table 2 Phenotypic characteristics of *Serratia plymuthica* isolates

No.	BIOLOG ID	Antifungal activity*		Haemolytic activity†	Production of prodigiosin
		Verticillium dahliae	*Rhizoctonia solani*		
s1	0·888	+++	++	+	–
s2	0·774	+++	+++	+	–
s3	0·759	+++	++	+	+
s4	0·897	+++	+++	+	+
s5	0·752	+++	+++	+	–
s6	0·876	+++	++	+	–
s7	0·879	++	+	+	–
s8	0·870	++	+	+	–
s9	0·793	+++	++	+	–
s10	0·863	+++	++	+	+
s11	0·726	+++	++	+	–
s12	0748	++	+	+	–
s13	0·962	++	+	+	+
s14	0·878	++	+	+	+
s15	0·800	+++	+++	+	–
s16	0·856	+++	+++	+	+
s17	0·800	+++	+++	+	+
s18	0·718	+++	+++	+	–
s19	0·899	+++	+++	+	–
s20	0·810	+++	+++	+	–
s21	0·664	+++	+++	+	–

*Antifungal activity against *Verticillium dahliae* and *Rhizoctonia solani* in dual culture assay; +++ represents > 10 mm wide zone of inhibition; ++ represents 5–10 mm wide zone of inhibition, + represents 0–5 mm wide zone of inhibition.
†Haemolytic activity on blood agar at 37 °C.

Table 3 Correlation between phenotypic and genotypic characteristics of *Serratia plymuthica* isolates

	Phenotype		Genotype		
No.	Biolog*	Antibiogram*	TGGE	BOX-PCR*	PFGE*
s1	I	I	I	I	I
s2	I	II	I	I	I
s3	II	II	I	II	II
s4	III	II	I	II	II
s5	III	II	I	III	VI
s6	III	II	I	III	III
s7	III	II	I	II	V
s8	I	II	I	II	V
s9	III	II	I	IV	VI
s10	III	II	I	IV	VI
s11	II	II	I	IV	VI
s12	III	II	I	IV	VI
s13	II	II	I	II	V
s14	III	II	I	II	V
s15	III	I	I	V	IV
s16	I	I	I	II	VIII
s17	II	I	I	II	VIII
s18	III	I	I	VI	IX
s19	III	I	I	VI	X
s20	III	III	I	VI	IX
s21	I	III	I	VI	VII

*Grouping on the basis of 75% similarity according cluster analysis (unweighted pair-group average).

ciated isolates showed haemolytic activity at 30 and 37 °C. Under the described conditions, only six isolates produced the red pigment prodigiosin with antifungal activity (Table 2).

All of the selected strains were resistant to several antibiotics. The strain s10 isolated from the rhizosphere of oilseed rape was most susceptible to antibiotics. This strain was only resistant to cefazolin and cefuroxim. In contrast, three isolates s16, s19, s21 were found with the resistance to eight antibiotics. The majority of the isolates have the same or a very similar antibiotic resistance profile. All of the strains were susceptible to piperacillin-tazobactam, cefuroxin, ceftacidim 1, imipenem, gentamicin, tobramycin, ofloxacin, cotrimoxazol, trimethroprim and nitroxolin. They were resistant against cefazolin and cefuroxin. The profiles were compared by numerical methods, and the resultant dendrogram based on percentage similarity between isolates showed the diversity. Three different clusters were found on the basis of 75% similarity (Table 3).

Three different methods were used to compare the isolates on the molecular level. PCR amplification of the 16S rDNA of each of the 21 isolates resulted in an amplification fragment (not shown). With DGGE it is possible to separate 16S rDNA fragments of the same length but of different sequences according to their melting properties. A linearly increasing denaturing gradient of urea and formamide in the presence of a constant temperature was used for separation of PCR products in DGGE. Each of the investigated isolates resulted in only one band with the same position on the gel (data not shown). The investigated *Ser. plymuthica* isolates could be distinguished from each other by using DGGE of the 16S rDNA fragments. According to their similarity all of them were grouped into the same group (Table 3). BOX-primed PCR was also used to differentiate the isolates. The PCR products obtained with BOX primers yielded DNA profiles with sufficient numbers of DNA bands to differentiate the 21 isolates (Fig. 1). The method was more discriminating than DGGE of the 16S rDNA, and various specific PCR fingerprints of *Ser. plymuthica* were found. Among them, BOX-PCR patterns of the potato-associated isolates (s18, s19, s20) were very similar to those obtained from the type strain of *Ser. plymuthica* (s21). The different BOX-PCR profiles were compared by numerical methods, and the resultant dendrogram based on percentage similarity between isolates showed a higher degree of genetic diversity than DGGE of the 16S rDNA fragment. At the 75% similarity level six major groups were defined (Table 3). Additionally, the determination of restriction fragment length polymorphisms by PFGE was used to compare the isolates. Use of the low-frequency restriction endonucleases *Spe*I for PFGE produced DNA profiles with several large DNA fragments for easy discrimination. This method was even more discriminating than BOX-PCR. Isolates with the same or a very similar BOX-PCR fingerprint, e.g. s19–s21 showed unique fingerprints in PFGE (Fig. 2). On the other hand, some isolates, e.g. s1 and s2 or s8 and s9 showed a very similar PFGE fingerprint. The different PFGE patterns obtained after digestion with *Spe*I were compared by cluster analysis. At the 75% similarity level 10 major groups were found (Table 3).

DISCUSSION

The BIOLOG system based on the oxidative utilization of 95 different carbon sources proved useful for the accurate identification of *Ser. plymuthica* strains. Grimmont *et al.* (1977) have demonstrated the usefulness of carbon source utilization tests for the identification of *Serratia*. The results of identification are in accordance with findings of previous studies using the API system (Kalbe *et al.* 1996). The plant-associated *Ser. plymuthica* isolates investigated in this study showed antifungal activities against the soilborne plant pathogens *Verticillium dahliae* and *Rhizoctonia*

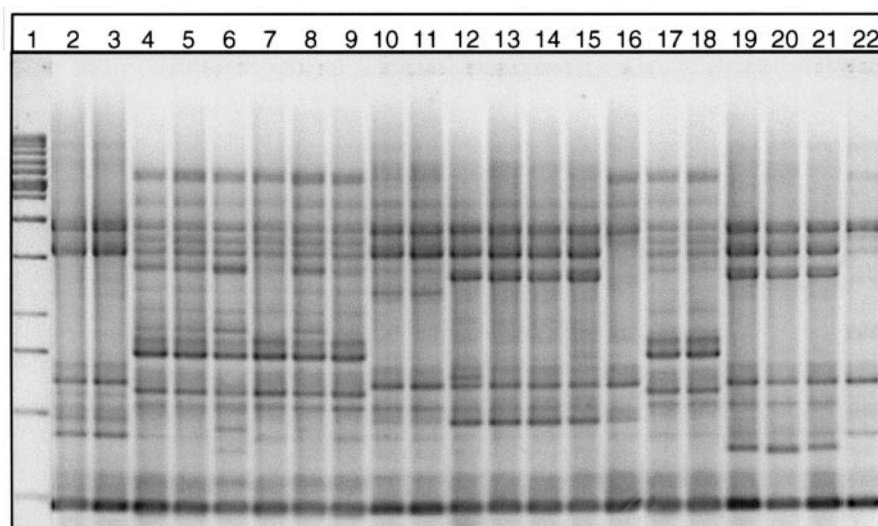

Fig. 1 Genomic BOX-PRC profiles of *Ser. plymuthica* strains. Lane 1: 1 kbp ladder, lane 2: s1, lane 3: s2, lane 4: s3, lane 5: s4, lane 6: s7, lane 7: s8, lane 8: s16, lane 9: s17, lane 10: s5, lane 11: s6, lane 12: s9, lane 13: s10, lane 14: s11, lane 15: s12, lane 16: s15, lane 17: s13, lane 18: s14, lane 19: s18, lane 20: s19, lane 21: s20, lane 22: s21 (type strain)

solani. Moreover, *Ser. plymuthica* has been used as biological control agent against fungal pathogens in agriculture such as strain s10 included in this study (Berg *et al*. 1998; Frankowski *et al*. 1998). The antifungal mode of action based on antibiosis, production of siderophores and the fungal cell-wall degrading enzymes chitinases and β-1,3 glucanases (Kalbe *et al*. 1996). The mode of action was strain-specific (Kalbe *et al*. 1996), e.g. for strain s10 the production of chitinases is responsible for antifungal activity (Frankowski *et al*. 1998). However, only six strains (= 29%) produced the non-diffusible red pigment prodigiosin. Pigmented biotypes of *Ser. plymuthica* were rarely isolated from plants (Grimmont and Grimmont 1992). Prodigiosin is an antifungal antibiotic and strains producing the pigment seem to be toxic to protozoa, and this may be an ecological advantage in the rhizosphere (Groscop and Brent 1964). The investigated plant-associated *Ser. plymuthica* strains showed haemolytic activity also at human-relevant temperatures. The haemolytic activity of different *Serratia* strains was investigated by Ruan and Braun (1990). One strain of *Ser. plymuthica* showed haemolytic activity and two genes *shl*A and *shl*B, which encode haemolytic poly-

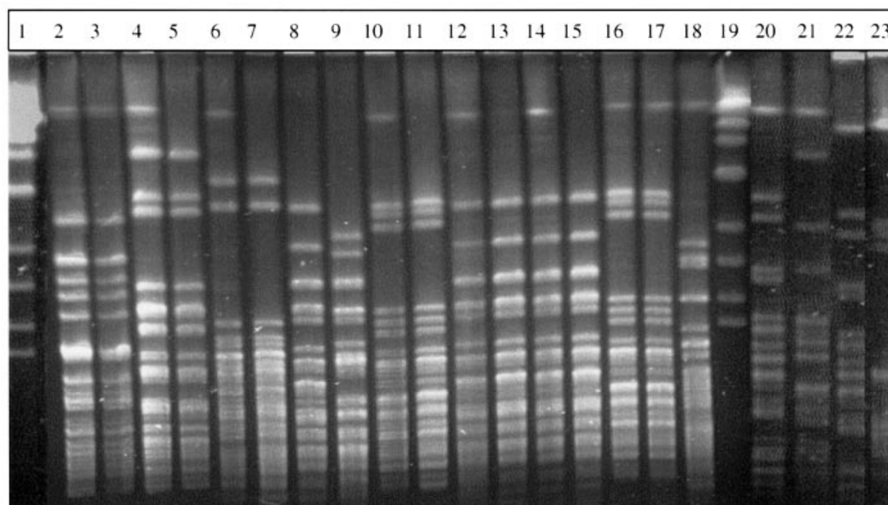

Fig. 2 DNA macrorestriction profiles of *Ser. plymuthica* produced by PFGE after *Spe*I digestion. Lane 1: lambda ladder marker (size range 225–1900 kbp), lane 2: s17, lane 3: s16, lane 4: s3, lane 5: s4, lane 6: s1, lane 7: s2, lane 8: s5, lane 9: s6, lane 10: s7, lane 11: s8, lane 12: s9, lane 13: s10, lane 14: s11, lane 15: s12, lane 16: s13, lane 17: s14, lane 18: s15, lane 19: lambda ladder marker (size range 225–1900 kbp), lane 20: s18, lane 21: s19, lane 22: s20, lane 23: s21 (type strain)

peptides were found (Ruan and Braun 1990). The investigated strains of *Ser. plymuthica* showed a low level of resistance to several antibiotics. This result is in contrast to other investigated multiresistant antagonistic rhizobacteria, e.g. *Serratia marcescens* (Grimmont and Grimmont 1992), *Stenotrophomonas maltophilia* (Berg *et al.* 1999) or *Burkholderia cepacia* (Wigley and Burton 1999). A correlation between the production of prodigiosin and the level of resistance as has been found in *Serratia marcescens* (Grimmont and Grimmont 1992) could not be found in *Ser. plymuthica*. The BIOLOG system and antibiotic resistance patterns as phenotypic typing methods showed the same discriminatory level. On the basis of 75% similarity it was possible to arrange the isolates into three groups according to both methods. On the other side, there was no correlation between the grouping.

Ser. plymuthica isolates were investigated on the molecular level using three different methods. The methods showed different discriminatory effects. The separation of the PCR-amplified 16S rDNA fragment in DGGE allowed a differentiation on the species level (Heuer and Smalla 1997). In our case, all the 16S rDNA fragments showed the same melting features. This is an indication, that all of the investigated isolates have an identical sequence of the 16S rDNA and belong to the same species. Therefore, this method did not prove useful to distinguish the isolates but to confirm their genetic similarity. BOX-PCR and PFGE were used as fingerprinting methods. BOX-PCR was less discriminatory than PFGE. Different discriminatory effects of molecular typing methods have also been described by other authors (Rademaker and De Bruijn 1992; Chetoui *et al.* 1998; Berg *et al.* 1999). Isolates in the same PFGE group are in each case closely related. They differ from each other by changes consistent with a single genetic event, i.e. point mutation or an insertion or deletion of DNA (Tenover *et al.* 1995). Isolates, which could not be differentiated by BOX-PCR showed unique fingerprints with PFGE. Some of the isolates (group I: s1, s2) are designated genetically indistinguishable because their restriction patterns have the same numbers of bands and the corresponding bands are the same apparent size. With PFGE, very high similarity was shown between some of the oilseed rape-associated strains. In conclusion, BOX-PCR and PFGE are useful fingerprinting methods for *Ser. plymuthica*. BOX-PCR was the fastest and least laborious method used. For patenting and also for epidemiological investigations, PFGE creates very specific fingerprints. On the basis of 75% similarity it was possible to arrange the isolates according to DGGE analysis of the 16S rDNA into one group, according to BOX-PCR into six groups and according to PFGE into 10 groups.

Relationships between the genotypic and the phenotypic properties were found. Strains s1 and s2, isolated from the rhizosphere of oilseed rape, were grouped into the same cluster according to the BIOLOG system, BOX-PCR and PFGE. They could only be distinguished by their antibiotic resistance pattern. The two prodigiosin producing strains s3 and s4 clustered together according to molecular patterns and antibiotic resistance patterns. Other examples are strains s18 and s19 isolated from the potato rhizosphere. They are very similar and could only be distinguished by their PFGE patterns. The type strain s21 was used as reference. This strain was grouped with each method together with other plant-associated strains included in this study. The strain could be distinguished by its PFGE patterns from the others. The data reported in this work are in agreement with other studies regarding correlations between phenotypic and genotypic methods to differentiate plant growth-promoting rhizobacteria like *Pseudomonas fluorescens* (Lemanceau *et al.* 1995) and *Burkholderia cepacia* (Di Cello *et al.* 1997, Wigley and Burton 1999).

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