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# Fast-Growing, Aerobic, Heterotrophic Bacteria from the Rhizosphere of Young Sugar Beet Plants

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**Fast-growing, aerobic, heterotrophic bacteria from the root surface of young sugar beet plants were inventoried. Isolation of the most abundant bacteria from the root surface of each of 1,100 plants between the second and tenth leaf stage yielded 5,600 isolates. These plants originated from different fields in Belgium and Spain. All isolates were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total cellular proteins. Comparison of protein fingerprints allowed us to inventory the bacteria of individual plants of different fields or leaf stages and to analyze the composition and variability of the rhizobacterial population of young sugar beet plants. Each field harbored a specific population of bacteria which showed a highly hierarchic structure. A small number of bacteria occurring frequently at high densities dominated in each field. The major bacteria were identified as *Pseudomonas fluorescens*, *Xanthomonas maltophilia*, *Pseudomonas paucimobilis*, and *Phyllobacterium* sp. The former three species showed a high genetic variability as they were represented by different protein fingerprint types on the same or different fields or leaf stages. Twinspan analysis and relative abundance plots showed that the structure and composition of the bacterial populations varied strongly over time. Pseudomonads were typically early colonizers which were later replaced by *X. maltophilia* or *Phyllobacterium* sp.**

During the last decade, much research has been directed toward the potential use of root-colonizing bacteria for the protection of crop plants against soil-borne pests and diseases or for direct promotion of plant growth (8–10, 14, 15, 23, 30, 35, 43, 47). Several reviews on so-called plant growth-promoting rhizobacteria have been published (4, 5, 19, 20, 34, 35, 40, 49). The inconsistent results with plant growth-promoting rhizobacteria inocula in field experiments stress that there is a need for fundamental research on all aspects of rhizobacterial plant growth promotion, e.g., proliferation, establishment, survivability on the root system, regulation of gene expression, and the role of different mechanisms in the overall influence of rhizobacteria on plant growth as well as on inoculum formulation.

To our knowledge, a large-scale inventory of free-living rhizobacteria to the strain level together with the analysis of their distribution among a relevant number of individual plants has never been performed, mainly because such analyses require a large amount of isolation, characterization, and identification work when classical methods are to be used. In the past, most studies have dealt with the distribution of either particular strains, species, and physiological groups (1, 7, 11, 25, 44) or total populations (24) of rhizobacteria. Some researchers have pooled together root pieces, root systems, or soil samples in order to reduce the amount of work (3, 29).

The use of fast and reproducible methods for strain characterization such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of total cellular proteins has facilitated the study of the diversity in natural populations of bacteria (12, 17, 22, 27, 31, 32, 36, 37, 41). Moreover, it is a practical, fast, and inexpensive technique which yields reproducible protein fingerprints allowing one to characterize and compare large numbers of isolates in a relatively short period of time.

Using this technique and one nonselective isolation medium, we have inventoried, at different growth stages, the flora of aerobic, heterotrophic bacteria occurring on the root surface of 1,100 sugar beet plants originating from different soils in Spain and Belgium. Such a study could provide us with specific information on the composition and diversity of this part of the rhizosphere flora.

## MATERIALS AND METHODS

**Sampling.** A total of 1,100 sugar beet plants were sampled on four different fields, three in Belgium and one in Spain (Table 1). The Belgian fields were sampled three times between the second and the tenth leaf stage. The Spanish field was sampled two times between the second and sixth leaf stage. Each sampling consisted of 100 plants collected in 10 sectors. On Belgian fields Hélécine (field A) and Pietsaer (field B) and the Spanish field Valladolid (field S) the 10 most vigorous plants from each of 10 field sectors, each consisting of 50 successive plants in one line were chosen. For the second and third sampling, sectors in the neighboring lines were chosen. The distance between the different sectors in each field was at least the length of a sector. All plants were sampled with a core of soil, packed in plastic bags, transported in ice chests, and processed in the laboratory within 24 h after sampling. In order to investigate whether there is a correlation between the presence of particular rhizosphere bacteria and differences in germination or growth rate of sugar beet seedlings, the four seedlings that germinated earliest, three seedlings that germinated later, and the three seedlings that germinated last were selected from each of the 10 sectors, at each of three samplings for the Belgian field Waasmont (field C).

**Isolation of rhizobacteria.** The entire root systems of individual plants were carefully washed with sterile tap

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TABLE 1. Sampling overview

| Location[a] | Sampling code | Sampling date | Leaf stage | Avg total population density (CFU/g of root) |
|---|---|---|---|---|
| Belgium | | | | |
| Hélécine | A1 | May 23 | 2 | $6 \times 10^7$ |
| | A2 | June 3 | 4 | $1 \times 10^8$ |
| | A3 | June 13 | 6, 7, 8 | $3 \times 10^7$ |
| Pietsaer | B1 | May 25 | 2 | $4 \times 10^7$ |
| | B2 | June 2 | 4 | $2 \times 10^7$ |
| | B3 | June 16 | 6, 7, 8 | $1 \times 10^7$ |
| Waasmont | C1 | June 17 | 6, 4, 2 | $5 \times 10^8$ |
| | C2 | June 23 | 8, 6, 4 | $8 \times 10^7$ |
| | C3 | June 27 | 6, 8, 10 | $4 \times 10^7$ |
| Spain | | | | |
| Valladolid | S1 | May 27 | 2 | $6 \times 10^7$ |
| | S2 | June 10 | 4, 5, 6 | $2 \times 10^8$ |

[a] The total sampled area was $3 \times 10^4$, $6 \times 10^4$, $5 \times 10^4$, and $4 \times 10^4$ $m^2$ on fields A, B, S, and C, respectively.

water to remove adhering rhizosphere soil. Root systems were then vigorously shaken for 15 min in a 50-ml tube containing 30 ml of a phosphate-buffered saline solution (Oxoid, Columbia, Md.) plus 0.025% Tween 20 (Sigma, St. Louis, Mo.) using a Stuart flask shaker (Stuart Scientific Co., Ltd., Great Britain). Two dilutions of the resulting suspensions were plated on agar plates containing 10% Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) plus 2% agar (TSBA) by using a Spiral plater (Spiral Systems Instruments, Inc., Cincinnati, Ohio). After incubation at 28°C for 2 days, one appropriate dilution plate (covered with 50 to 300 colonies per plate) per plant was used for colony counts and isolations. Total counts and counts of dominant (i.e., most abundant) colony types were made. Preliminary experiments have shown that morphologically identical colonies on TSBA yield an identical protein pattern in SDS-PAGE (see below). One colony of each of the five most abundant colony types was isolated and transferred to a well in each of two 48-well tissue culture clusters (Costar Europe, Ltd.) containing 500 μl of TSB (TSBA without agar). This procedure was repeated for each plant. On the average, the isolated colonies represented 80% of all colonies on the TSBA plate. The tissue culture clusters were incubated at 28°C for a standardized period of exactly 48 h. Glycerol was added to fully grown cultures (final concentration, 25% [vol/vol]). One cluster was stored at −70°C. The second cluster was used for further strain characterization.

**Characterization of rhizobacteria by SDS-PAGE.** The tissue culture clusters containing the fully grown isolates were centrifuged for 30 min at 300 × *g* (fixed angle) by using special swinging adapters. The supernatants were discarded. Each pellet was preincubated for 15 min at 37°C in 10 μl of a lysozyme solution (1 mg/ml). Total cellular proteins were extracted by boiling each pellet at 95°C for 10 min in 50 μl of sample buffer mix (2.5% sodium dodecyl sulfate, 0.125 M β-mercaptoethanol, 150 mM Tris [pH 8.8], 4 mM EDTA, 0.75 M sucrose, 0.075% bromophenol blue) and sonicating for 10 s. After being cooled on ice, 7 μl of a 0.5 M iodoacetamide solution was added. The extracted total cellular proteins were separated by electrophoresis in polyacrylamide gels and visualized by Coomassie blue staining as described previously (22). The protein fingerprints were standardized photographically. The photographs of the fingerprints were compared and sorted in fingerprint types (FPTs) which were numbered sequentially. An FPT is defined here as a set of bacterial isolates with identical, photographically standardized protein patterns, obtained via one-dimensional electrophoresis of denatured total cellular protein extracts of standardized cell cultures and detection of the separated proteins by Coomassie blue staining.

**Identification of FPTs.** FPTs were identified as described previously by classical biochemical tests in combination with API identification kits (22). Unpublished experiments in our laboratory based on conventional identification methods have demonstrated that isolates with identical SDS-PAGE patterns belong to the same species or subspecies. Hence, we identified only two randomly chosen isolates per FPT. Only the most important FPTs were identified.

**Data analysis.** The colony counts allowed us to calculate, for each FPT, its absolute density on each individual plant. The absolute density is expressed as the number of colony-forming units per gram of root, a measure that is currently used in rhizobacteriology, but that does not necessarily reflect the ecological density, i.e., the real density in the available volume of niche. However, the latter is difficult to determine. The frequency of occurrence of an FPT is the percentage of plants on which it was found.

To look for any pattern in the data and to investigate the similarities between the samples, a Twinspan analysis (two-way indicator species analysis) was carried out. Twinspan analysis is a polythetic divisive clustering technique (13) based on a reciprocal averaging ordination (16). Twinspan analyses were performed based on all individual plants and FPTs per field and per sampling and were done on all individual plants and FPTs per field for the two or three samplings together. As plants of any given sector, in general, clustered together, further analysis was carried out per sector based on the average density of each FPT. This was done per field, for all samplings together, and for all samplings of all fields together. In the latter case, only those FPTs that were present on more than 10% of the plants at a leaf stage or sampling (at least one) were included in the Twinspan. The cut levels used followed a lognormal series (0, 1, 4, 16, 64, 256, 1,024, 4,096 [$10^6$ CFU/g of root]).

The pattern of distribution of organisms is often described by means of the frequency distributions of the number of organisms found per sample. However, this pattern is an unstable aspect of a dynamic system. A better method to describe the spatial aggregation of FPTs in a field is the Taylor power function (45) ($V = a \times m^b$ or $\log V = \log a + b \times \log m$.) which states that the variance ($V$) is proportional to a fractional power of the mean $m$. The exponent $b$ is a measure of aggregation and has continuous values ranging from zero for a uniform pattern, one for a random pattern, and two for a situation of maximum contagion (all organisms in one sample). The exponent is useful to describe and compare the aggregation of populations on different fields and over different periods (28). It was calculated by least-squares linear regression analysis based on the mean and variance per location and per sampling date.

The correlation between frequency of occurrence and absolute densities was expressed as the product-moment correlation coefficient.

Spearman correlation coefficients between FPTs per field were calculated to express the association or complementarity of particular FPTs within a field (42).

TABLE 2. Dominant FPTs from sugar beet[a]

| Species | FPT | $F_T$ | $F_{ABC}$ | $F_A$ | $F_B$ | $F_C$ | $F_S$ |
|---|---|---|---|---|---|---|---|
| *Pseudomonas fluorescens* | 1 | 24 | 28 | 52 | 18 | 15 | 5 |
| *Phyllobacterium* sp. | 3 | 18 | 14 | 14 | 12 | 13 | 36 |
| *Xanthomonas maltophilia* | 2 | 15 | 18 | 33 | 2 | 19 | 0 |
| *Pseudomonas fluorescens* | 5 | 11 | 6 | 1 | 14 | 1 | 35 |
| *Xanthomonas maltophilia* | 9 | 11 | 12 | 5 | 21 | 12 | 3 |
| *Pseudomonas paucimobilis* | 7 | 9 | 3 | 3 | 6 | 0 | 36 |
| *Pseudomonas fluorescens* | 6 | 8 | 10 | 3 | 7 | 18 | 1 |
| *Xanthomonas maltophilia* | 4 | 8 | 1 | 2 | 1 | 0 | 37 |
| *Xanthomonas maltophilia* | 15 | 7 | 8 | 9 | 4 | 10 | 2 |
| *Pseudomonas fluorescens* | 13 | 6 | 6 | 9 | 4 | 5 | 6 |
| *Pseudomonas paucimobilis* | 23 | 6 | 7 | 5 | 12 | 4 | 0 |
| *Xanthomonas maltophilia* | 19 | 5 | 6 | 11 | 1 | 7 | 0 |
| *Xanthomonas maltophilia* | 8 | 5 | 6 | 0 | 18 | 0 | 3 |
| *Pseudomonas fluorescens-putida* | 104 | 5 | 5 | 2 | 7 | 6 | 5 |
| *Pseudomonas fluorescens* | 10 | 4 | 5 | 2 | 14 | 0 | 1 |

[a] The 15 most frequent FPTs are listed together with total frequency ($F_T$), frequency in Belgium ($F_{ABC}$), frequency on fields Hélécine-Belgium ($F_A$), Pietsaer-Belgium ($F_B$), Waasmont-Belgium ($F_C$), and Valladolid-Spain ($F_S$). Frequencies give the percentage of plants on which the indicated FPTs were found. The FPT number gives the (arbitrary) FPT designation as it was given during the study. The total number of FPTs was 485.

## RESULTS

This study has focussed on the population of fast-growing, aerobic, heterotrophic bacteria of the root surface of sugar beet plants which will hereafter be designated the rhizobacterial population.

**Isolation and characterization.** A total of 5,612 isolates were obtained from 1,100 sugar beet plants. Only 4,709 gave fingerprints of a sufficient quality for comparisons and sorting into FPTs. All FPTs were numbered. After comparison, we noticed that some strains had been isolated two or more times from the same plant although they were considered as different colony types. This is due to differential growth rates which cause colonies to be different in size. All the double isolates were eliminated, which left us 4,081 independent isolates from 1,100 plants, representing 485 FPTs. All FPTs showed characteristic, distinctive, and reproducible patterns.

**Overall distribution of rhizobacterial FPTs.** The majority of FPTs (417) were found on 1 to 11 of the 1,100 plants and accounted for 1,968 isolates (42% of the total number). The remaining 68 FPTs were found on 12 to 264 plants (>1% of the plants). They make up 2,741 isolates (58% of the total number). In Table 2, the 15 most frequently occurring FPTs and their identity are listed by decreasing total frequency of occurrence.

**Comparison of the rhizobacterial population of plants with differential germination rates.** At Waasmont (C), we have investigated the rhizobacterial population of plants with different germination rates. Twinspan classification of FPTs and individual plants (data not shown) from the first sampling (C1) showed that plants cluster in 12 groups: four groups (making up 81% of the plants) harbor plants of all three leaf stage classes. Other cluster groups contain plants of one or two leaf stages. Thus, plants with a different growth rate harbored a similar rhizobacterial population. Similar results were obtained for the other samplings (C2 and C3). Consequently, it is very unlikely that germination rate differences are caused by differences in the composition of the population of aerobic, heterotrophic bacteria.

**Rhizobacterial population within a field.** Only a minority of FPTs was found frequently, while the majority was only occasionally detected. The product-moment correlation coefficient between frequency of occurrence and average absolute density of FPTs (per field and sampling date) varied between 0.45 (B1) and 0.98 (S2) ($51 \leq n \leq 118$; $P < 0.0001$ in each case). This significant positive correlation between frequency of occurrence and absolute density also holds after exclusion of outliers. This means that FPTs which were found over many plants were also present at higher densities than rare FPTs. This implies that each field is dominated by a relative small number of FPTs. In order to determine whether the difference in frequency of occurrence or density per plant was not due to an experimental factor, e.g., a difference in colony-forming or growth capacity on TSBA or antibiosis on this medium, the colony-forming capacities of the rare FPTs and frequent FPTs were determined and found to be equal. Moreover, no antibiosis was observed in reconstruction experiments whereby known numbers of cells of different FPTs were inoculated on the same plates and the relative number of colonies counted. This indicates that the observed correlation cannot be due to an intrinsic experimental factor.

The high variability of total population densities (Table 1) and densities of individual FPTs (data not shown) indicates that the rhizosphere bacteria that we studied occur in an aggregated pattern or that there is a low probability of finding them at similar densities in each sample. A better method to describe aggregation patterns of populations is the *b* value of the Taylor power function. When calculated for the 10 most frequent FPTs, nearly all *b* values were significantly greater than one. Thus, FPTs attain significantly higher densities on particular spots in the field. Spearman correlation coefficients between several frequent FPTs showed that only in a few cases was there a significant positive correlation, between FPT 5 (*Pseudomonas fluorescens*) and 7 (*Pseudomonas paucimobilis*) at S1 and FPT 3 (*Phyllobacterium* sp.) and 4 (*Xanthomonas maltophilia*) at S2. These bacteria mostly coexisted on the same plants and did not outcompete each other. No other significant positive or negative correlations were observed. This shows that the clusters of different FPTs occurred all over the field and were not all concentrated in particular areas. This was confirmed by the Twinspan analyses of individual plants and FPTs per field and sampling: the cluster groups of plants contained, in general, plants of the same sector, showing that plants of a same sector harbored similar bacterial communities which differed from other sectors.

**Geographical and early seasonal variability. (i) Variability of the structure of the rhizobacterial population.** The structural composition of the population on fields C and S and its evolution with time can be deduced from Fig. 1. The plots of fields A and B were analogous to the ones of C and S, respectively. The typical sigmoidal curve is found when only a few species are very abundant, while many species are present at intermediate or low densities (Fig. 1). The more vertical the curve is, the more hierarchic, and the more horizontal the curve is, the more egalitarean the community is. The plots show that the structural composition of the bacterial population may evolve differently with time on different locations. At Waasmont (C) and Hélécine (A), the slopes of the curves are more or less similar for the three samplings, whereas at Valladolid (S) and Pietsaer (B), the curves become much steeper at later leaf stages, indicating the development of a more hierarchically organized population.

**(ii) Variability of the composition of the rhizobacterial population.** The previous analyses show changes in the

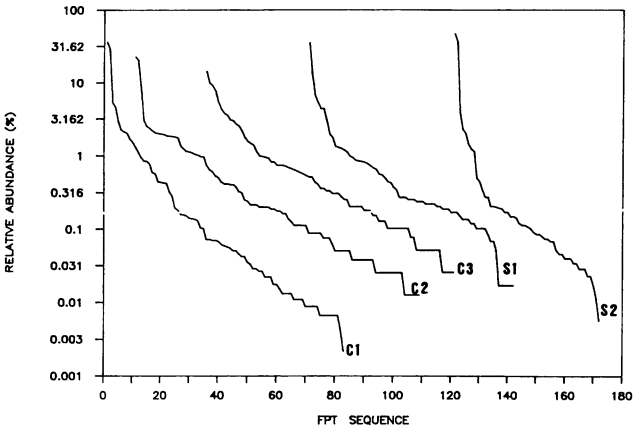


FIG. 1. Patterns of FPT relative abundance. The plots present the logarithm of the percentage that each FPT represents on the sum of the total number of bacteria of all plants of each field in function of the FPT sequence (ordered from the most to the least abundant). Patterns are given for all samplings at Waasmont, Belgium (C1, C2, and C3) and Valladolid, Spain (S1 and S2).

structure of the population but give no information on the variability of their qualitative composition. In order to compare the similarity of the rhizobacterial population of plants of different fields and different leaf stages, we have carried out a Twinspan analysis. Twinspan analyses of individual plants and FPTs per field for the two or three samplings indicated a high variability with time. As mentioned earlier, the Twinspan analyses of individual plants per field and per sampling showed that the rhizobacterial population of plants of each sector were very similar but different from other sectors. Hence, the overall Twinspan analysis (Fig. 2) was calculated based on the average FPT densities per sector instead of per plant. At the first division, all sectors from S and the majority of B are split from those of A and C. In a further division, all sectors of B and S and most sectors of A and C are split in different groups. Thus, sectors from the same fields cluster mainly together and apart from others. Therefore, each field seems to harbor a rather specific rhizobacterial population. Nearly all sectors of C1 cluster together with cluster group 3 of field A, and some sectors of the early leaf stages of field B (B1 and B2) belong to the same cluster group 4 of field A. Hence, the composition of the early communities of fields B and C are similar to the ones of field A. On fields C and S, there is a different population at the different leaf stages; the sectors of these two fields cluster according to the samplings or leaf stages. On the other fields, there was no significant difference between the population at different leaf stages since the cluster groups (groups 3, 4, and 5) were composed of sectors from all samplings.

**(iii) Population constituents on different fields and at different leaf stages.** The constituents of the population on the different fields and leaf stages can also be deduced from Fig. 2. Although *P. fluorescens*, *P. paucimobilis*, *Phyllobacterium* sp., and *X. maltophilia* were present on all fields, they were often represented by different FPTs, with average densities varying strongly at different leaf stages, e.g., *P. fluorescens* FPT 1 at C1 and FPT 55 at C3. Hence, different FPTs are often found in different FPT cluster groups.

At Hélécine (A), there was a gradual qualitative change over a 20-day period (23 May until 13 June). At the second leaf stage, the major components of the rhizobacterial populations were the *P. fluorescens* FPTs 1 and 13 and the *X. maltophilia* FPTs 15 and 19. *P. fluorescens* FPT 1 was constantly found on ±50% of the plants in the whole investigated period. The three other early colonizers were becoming less frequent later, and other FPTs were found more frequently near 13 June, i.e., *P. paucimobilis* FPT 50, *Phyllobacterium* FPT 3, and *X. maltophilia* FPT 2. The latter FPT exhibited an explosive development: on 23 May (A1) it was found on 3% of the plants at an average density of $3 \times 10^5$ CFU/g root but on 13 June (A3) it occurred on 58% of the plants at an average density of $14 \times 10^6$ CFU/g of root, a 50-fold average increase over a 10-day period!

At Pietsaer (B), the rhizobacterial population was quite heterogenous, as indicated by the fact that several sectors from the same field are separated at the first Twinspan division (Fig. 2B). At the fourth leaf stage (B2), the rhizobacterial population was similar to those at the second leaf stage (B1); two Twinspan groups each comprise sectors from both samplings. At the second leaf stage (B1), the dominant bacteria were *P. fluorescens* FPTs 1 and 10, *X. maltophilia* FPT 9, followed by some unidentified FPTs. Seven days later, FPT 10 and FPT 9 were found on 26 and 28% of the plants, respectively. At the third sampling (B3), the composition of the population had changed somewhat; the major types were *X. maltophilia* FPT 8, replacing *X. maltophilia* FPT 9, and *Phyllobacterium* FPT 3. *Pseudomonas* strains were typical early colonizers, which were later replaced by *X. maltophilia* and *Phyllobacterium* sp.

At Waasmont (C), the rhizobacterial population was characterized by spectacular qualitative and quantitative fluctuations within a 10-day period, which is illustrated by the fact that nearly all sectors from each sampling period clustered together in different groups. At the first sampling (C1), *X. maltophilia* FPT 2, *Phyllobacterium* FPT 3 and *P. fluorescens* FPT 1 (21% of the plants) were predominant. Six days later (C2), only *P. fluorescens* FPT 1 was still found on 22% of the plants but other types were now predominant. On 27 June (C3, 10 days after the first sampling) the three earlier mentioned *X. maltophilia* FPT 2, *Phyllobacterium* FPT 3, and *P. fluorescens* FPT 1 were only rarely found and several other FPTs were appearing.

The most spectacular fluctuations occurred on the Spanish field (S). *P. paucimobilis* FPT 7 and *P. fluorescens* FPT 5, which were found on 70 and 62% of the plants (S1) at an average density of $2 \times 10^6$ and $2 \times 10^7$ CFU/g of root, respectively, had nearly disappeared 14 days later (S2). On 10 June (S2), *Phyllobacterium* FPT 3 and *X. maltophilia* FPT 4 were both found on 76% of the plants at an average density of $6 \times 10^7$ and $9 \times 10^7$/g of root, respectively. The rhizobacterial population changed and became more diverse. Also, the structure became more hierarchic, as shown earlier. All sectors of S1 are found in one cluster, while those of the second sampling are divided in two clusters.

## DISCUSSION

In this study, we have made an inventory of an important part of the community of rhizosphere bacteria of young sugar beet plants. We realize that the conclusions reached cannot be extrapolated to the whole rhizobacterial community on every sugar beet plant. Indeed, we selected only the most vigorous plants for our work as our underlying purpose was to find out which bacteria are associated with such plants. The second limitation of the present study is that only one isolation medium was used. We used 10% TSBA, which, according to our previous study (22), yields only 28% of the

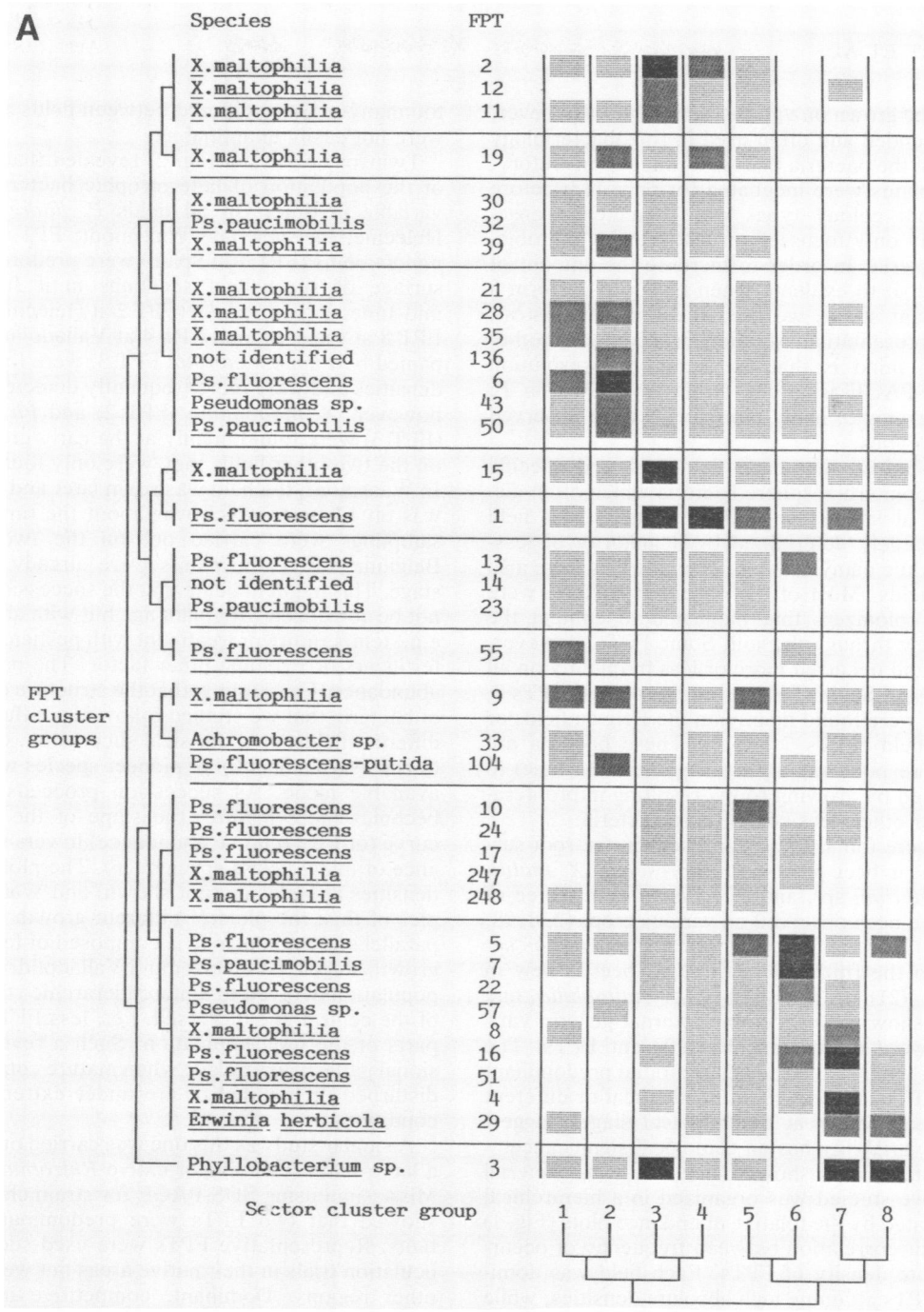


FIG. 2. Classification of FPTs and field sectors from all samplings by Twinspan analysis. The resulting two-way table and dendrograms are given. Shading in the table gives the average density per FPT for that cluster of sectors. Only FPTs that occurred on more than 10% of the plants at a leaf stage (at least one) were included. The table in Fig. 2B gives, per sampling, the number of sectors that belong to the indicated cluster groups.

bacteria that can be grown on various agar media. However, TSBA is recommended and often used in rhizobacteriology for the isolation of the bacteria that we wanted to inventory. In addition, the plates were incubated for only 48 h before the most abundant colony types were detected. Consequently, this study only focussed on fast-growing, aerobic, heterotrophic bacteria. In order to increase the amount of samples that we had to evaluate when trying to perform a statistically relevant study, we used the simplest, fastest, and the most representative isolation procedure. Another point to keep in mind is that the taxonomic resolution reached by SDS-PAGE is at the strain level. Within *P. fluorescens*, for example, many more FPTs were observed than there are biotypes.

The analysis showed that each field harbored specific aerobic, heterotrophic rhizosphere bacteria which often disappeared rapidly at later stages. The specificity of a field population was largely determined by a majority of less-frequent FPTs, while many of the dominant FPTs were also found on other fields. Most of the observed bacteria were probably casual colonizers that happened to contact the roots as these grew through the soil. Some FPTs, however, were ubiquitous and occurred more or less frequently on all fields in Belgium and Spain (FPTs 1 and 3); others (FPTs 9, 6, 23, 19, and 15) were found mainly on the Belgian fields or on the Spanish field (FPTs 7 and 4). These bacteria are probably able to respond rapidly (short generation time) to the energy released by growing roots, resulting in proliferation at the cost of other, less competitive bacteria.

Four species were found to be dominant on the root surface of young sugar beet plants: *P. fluorescens*, *X. maltophilia*, *Phyllobacterium* sp., and *P. paucimobilis*. Three of these species have been observed on various crops (2, 6, 18, 22, 38, 39, 46, 48), but the discovery of *Phyllobacterium* sp. (*Rhizobiaceae*) in the rhizosphere of sugar beet is new in rhizobacteriology (21). *P. fluorescens*, *X. maltophilia*, and *P. paucimobilis* show a considerable internal genetic variability as they were represented by different FPTs. The observations that within each species we found predominant as well as rare FPTs and that FPTs of identical or different species replace each other at different leaf stages suggest that this genetic variability has an ecological significance.

The present study also showed that the rhizobacterial population that we studied was organized in a hierarchical way as demonstrated by the relative abundance plots (Fig. 1) and the significant correlation between frequency of occurrence and absolute density of FPTs. Each field was dominated by a few FPTs attaining high absolute densities, while the majority of FPTs were rarely found and occurred at lower densities. The densities of FPTs were highly variable, not randomly distributed but aggregated, which was confirmed by the Taylor power indices. The pattern of a few common organisms at high densities and many rare species at low densities is characteristic in classical community studies (33). Predominant FPTs showed an aggregated pattern and attained higher densities on certain microsites in the field. Clusters of FPTs were spread all over the field. Thus, each microsite might have clones (ecotypes) adapted to its particular set of environmental conditions. In a recent study, McArthur et al. (27) proved that several different clonal populations of the soil bacterium *Pseudomonas cepacia* existed along a landscape gradient and that the observed genetic diversity was positively correlated with the variability of certain environmental parameters. In our study, we have not tried to correlate the characteristics of the bacterial populations of a field and environmental parameters, since too many of these differed between fields and some of them were not easily quantifiable.

Twinspan analysis (Fig. 2) revealed that the composition of the population of heterotrophic bacteria may be highly variable in time. On all fields *P. fluorescens* (FPT 1 at Hélécine, Pietsaer, and Waasmont; FPT 5 in Spain) or *P. paucimobilis* (FPT 7 in Spain) were predominant on the root surface of sugar beet seedlings until June. Later, near mid-June, *X. maltophilia* (FPT 2 at Hélécine and Waasmont, FPT 8 at Pietsaer, and FPT 4 at Valladolid), mostly accompanied by *Phyllobacterium* FPT 3, were found at increasing densities and were more frequently detected. At Waasmont, however, *X. maltophilia* (FPT 2) and *Phyllobacterium* sp. (FPT 3) were found mainly at the early growth stage, while on the two other fields they were only found at later stages. In Waasmont, the field was sown later and the first sampling was on 17 June, which was about the time when the third samplings were carried out on the two other fields in Belgium, where the plants were already at the sixth leaf stage. This might indicate that the successional pattern might not be correlated with plant age but with abiotic parameters, e.g., temperature or treatment with pesticides, herbicides, or fertilizers or by some other factor. The patterns of relative abundance (Fig. 1) show that the structure of the populations of bacteria that we studied followed a different evolution on different fields. In a classical succession, communities tend to consist of a handful of pioneer species which colonize the available niche. As succession proceeds, the community becomes more mature, the slope of the typical S-shaped curve (of FPT relative abundance) lowers with a preponderance of middle class species (26). The plots of relative FPT densities at Hélécine (not shown) and Waasmont are examples of this; the plots of different growth stages are almost parallel and the top zone is composed of fewer FPTs at later growth stages. At Pietsaer and Valladolid, the rhizobacterial population evolved to a more hierarchic structure; the slope of the central line increased, i.e., less FPTs made up larger parts of the total population. Such a reversal of a successional pattern takes place when mature communities become disturbed or polluted or are under extreme environmental conditions.

A similar study as this one was carried out by Paau (36). In a large-scale screening of native *Rhizobium* strains in West Mississippi using SDS-PAGE for strain characterization, he showed that two FPTs were predominant nodule inhabitants. Representative FPTs were used successfully in reinoculation trials in their native areas but were not effective in other regions. Dominant, competitive indigenous strains isolated from one geographical location will remain competitive in that area but might not be competitive when applied to a totally different region. Plant growth promotion by microorganisms will largely depend either on the composition and application of mixtures of microorganisms each adapted to particular conditions or on the application of a highly adapted microorganism, selected for particular conditions. Our study might be valuable for the selection of strains to be used for the study of plant growth-promoting bacteria for sugar beet plants.

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