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THE BACTERIAL FLORA IN AND OUTSIDE THE RHIZOSPHERE OF WHEAT, GRASS, SUGAR BEET AND POTATO ON A LIGHT SANDY LOAM

by

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1. INTRODUCTION

Hiltner (1904) discovered that the immediate vicinity of plant roots was much richer in microbes than the soil further away from a root. He called the zone in the soil influenced by a root the rhizosphere.

The comprehensive work of Starkey (1929[a+b]) was stimulating for many others. The quantity of bacteria, but also of other organisms in the rhizosphere microflora differed from that of the soil. The rhizosphere contained other morphological groups of bacteria than the soil (Lochhead, 1940), which had different properties with regard to Gram's staining method and their nutritional requirements (Lochhead and Thexton, 1947; Wallace and Lochhead, 1949; Kaunat, 1961). The composition of the bacterial flora of the rhizosphere was found to be different for different plants (Wallace and Lochhead, 1949; Rouatt and Katznelson, 1961; Kaunat, 1963) and presumably also for the different stages of plant development (Starkey, 1929[b]; Wallace and DeKing, 1954).

Reviews by Starkey (1958) and Rovira (1965) show that many investigators confirmed the observations of the early workers and extended the field of study. Much valuable information has also been given by Macura and Vančura (1965).

Vágnerová *et al.* (1960) thoroughly studied the morphological properties and nutritional requirements of the bacterial flora in rhizosphere soil of young and old wheat plants. Besides, the generic composition in the rhizosphere of young plants was determined and compared with that of soil not influenced by living plant roots.

Our aim was to study the generic composition of the microflora of the rhizosphere of four crops and of the corresponding non-rhizosphere soil. When we started in 1969, knowledge of the generic composition of the microflora of soil and rhizosphere soil was limited, especially with regard to bacteria and actinomycetes.

The fungal flora was studied by Van Emden (1972), the actinomycetes by Vruggink (1976), and we studied the bacteria in samples taken from the same plots on the same dates.

2. MATERIALS AND METHODS

The crops were grown on a small experimental crop rotation field of the Plant Protection Service in the polder Oostelijk Flevoland. The soil was a homogeneous light sandy loam (8-10% particles < 20 µm) and still contained many shells and shell fragments. The pH was 7.6 (in KCl).

Samples were taken from small plots (4 × 4m) on each of which the crops - wheat (usually winter wheat), grass (*Lolium multiflorum*, var. Westerwoldicum), sugar beet and potatoes - had been grown for six years in succession after the polder had been reclaimed.

Sugar beet tops were removed; the grass and the wheat stubble were ploughed down.

The soil was sampled in March, May, June, July and October 1969 to a depth of 15 cm with a 2 cm auger. Cores were taken between the rows of plants. Five plants plus adhering soil were taken for rhizosphere studies. In the case of grass, parts from rows with a total length of about 25 cm were taken.

The preparation of the dilutions, the composition of the media used for counting, the isolation and pure culturing of isolates and the way of determining their properties with regard to cell morphology, reaction to Gram's stain, motility, flagella staining and ability to decompose or to convert certain substrates have been described by Velvis (1975).

For the determination of the genus we used for Gram-negative bacteria the scheme of Hendrie *et al.* (1964), for Gram-negative and -positive soil bacteria the scheme of Rivière (1963), further Skerman's "Guide to the identification of the Genera of Bacteria" (1959) and the seventh edition of Bergey's "Manual of Determinative Bacteriology" (Breed *et al.*, 1957). A scheme is, however, only a simplification of reality; a map, with which one can work quite well when isolates fit into it. Deviations from the scheme or exceptions to it, if an isolate does not quite fit into a well defined genus or if it belongs to a group of ill-defined genera as is the case with the "coryneform bacteria" (*Arthrobacter*, *Corynebacterium*, *Brevibacterium*, *Mycobacterium*, *Nocardia* and related genera, not normally found in soil), cause difficulties.

3. RESULTS

3.1. Numbers of bacteria

The total numbers of bacteria (viable counts) in soil and rhizosphere in March, May, June, July and October and sometimes the number of casein and starch hydrolyzing bacteria plus the number of *Azotobacter* are presented in table I.

TABLE I. Bacterial numbers in soil (S) and rhizosphere (R) of sugar beet, potato, wheat and rye grass of a light sandy loam in Oostelijk Flevoland at different dates.

	Beet		Potato		Wheat		Grass	
	R	S	R	S	R	S	R	S
3/18								
total numbers (x 10^6)		18 ± 9		51 ± 10		19 ± 6		25 ± 7
casein decomp. (x 10^5)		35 ± 8		46 ± 9		47 ± 14		35 ± 8
5/5								
total numbers (x 10^6)		10 ± 5		6 ± 3	148 ± 50	16 ± 5	40 ± 19	57 ± 11
casein decomp. (x 10^5)		26 ± 5		26 ± 5	570 ± 90	41 ± 6	130 ± 70	39 ± 6
starch decomp. (x 10^5)		7 ± 1		13 ± 3	210 ± 30	15 ± 2	140 ± 70	20 ± 2
6/30								
total numbers (x 10^6)	38 ± 12	5 ± 1	99 ± 21	15 ± 4	15 ± 2	7 ± 3	17 ± 3	13 ± 5
casein decomp. (x 10^5)	15 ± 10	9 ± 4	21 ± 10	9 ± 4	16 ± -	9 ± 3	42 ± -	22 ± 5
starch decomp. (x 10^5)	130 ± 30	52 ± 10	220 ± 20	51 ± 10	120 ± 20	50 ± 10	120 ± 10	56 ± 10
Azotobacter (x 10^2)	5.6 ± 2	2.4 ± 0.4	67 ± 5	11.3 ± 2.6	5.2 ± 2	2.5 ± 0.5	1.8 ± 0.2	1.3 ± 0.2
7/21..								
total numbers (x 10^6)	109 ± 6	5 ± 1	286 ± 12	19 ± 4	66 ± 5	16 ± 4	95 ± 9	11 ± 4
10/6								
total numbers (x 10^6)		20 ± 3		25 ± 3		23 ± 3		20 ± 4

The numbers are expressed per gram of oven-dry soil (105 °C).

On 18 March the only crop was wheat. The number of bacteria (per gram of dry soil) was similar in soil on which during the previous year sugar beet, grass and wheat were grown; in the case of potatoes it was about twice as high. The number of casein hydrolyzing bacteria did not show differences. The number of *Azotobacters* was below 1000 per gram of dry soil.

On 5 May grass and wheat were present; sugar beet and potatoes had not yet emerged. In the soil between the rows of wheat and grass plants the total number of bacteria was higher than in soil of the beet and potato plots. A clear rhizosphere effect was shown by wheat. A typical results were obtained for grass, as soil showed higher numbers than rhizosphere. The casein hydrolyzing bacteria showed slightly higher numbers in soil (between te rows) of the grass and wheat plot than in that of the beet and potato plot. In both crops a clear rhizosphere effect was present. The number of starch hydrolyzing bacteria was not very different in soil from the four plots. A rhizosphere effect was clear for wheat and grass. The number of *Azotobacter* was lower than 1000 per gram dry soil.

On 30 June all crops were present and the total numbers of bacteria showed clear rhizosphere effects for beet and potato and hardly for wheat and grass. The numbers in soil were low, especially between the beet rows where the soil was open to insolation and very dry.

The number of casein hydrolyzing bacteria was lower than before and that of the starch hydrolyzing higher. Only the latter showed a clear rhizosphere effect.

In the soil and rhizosphere of the potato plot the numbers of *Azotobacter* were higher than 1000 per gram of soil. The soil between the rows seemed to be influenced by the roots.

Because the numbers of the total counts of 30 June were too low for making isolations, new counts at the correct dilutions were made on 21 July. The numbers in soil were still low. The rhizosphere effect was clear.

On 6 October the soil was plowed; the grass and the wheat stubble were plowed down, the other crops had already been removed. At that time the total numbers of bacteria in the different plots were similar.

The total number of bacteria in non-rhizosphere soil seems to be lowest in summer, probably due to lack of food.

A part of the small colonies isolated from the original plates died quite rapidly after the first transfer. Others disappeared more slowly and lost mass after each transfer until finally a haze of cells was left, which did not show any further growth. This is presumably due to the fact that the media used lacked one or more growth substances required by these

isolates. The lost isolates may be considered as pure. (The disappearance of a species from a mixture is normally not noticed.)

Unfortunately many isolates and samples were lost due to a fire. Among them were all isolates from the first sampling. From other samplings some series were reduced so seriously that it did not make sense to use them further.

The properties of pure cultured isolates of some series according to their behaviour towards *Gram's staining* method and their *morphology* are presented in Appendix I. Information about the isolates of the second, third and fourth sampling of the following series is presented: grass plot, soil (Gs) and rhizosphere (Gr); potato plot, soil (Ps) and rhizosphere (Pr); beet plot, soil (Bs) and rhizosphere (Br); and wheat plot, soil (Ws) and rhizosphere (Wr). The number of the sampling is following the letters. So Wr_2 means: wheat rhizosphere at the second sampling.

Gram's stain. At the time of the second sampling the plants (grass and wheat) were still only small, and the soil was rather moist. The majority of the bacteria in the rhizosphere (Wr_2 and Gr_2) proved to be Gram-negative (G-), while in the soil (not influenced by plant roots) the majority was Gram-positive (G+) and Gram-variable ($G^+/_-$).

The third sampling showed a different picture: the majority of the bacteria in the rhizosphere and in the soil was now Gram-positive or Gram-variable. Quantitatively the rhizosphere effect still existed (table I), but the qualitative change is striking.

This concerns all samples of which bacterial isolates were obtained in pure culture. No correction is given for lost strains.

It is clear that between the populations of bacteria in the rhizosphere and soil quantitative and qualitative differences exist, and that qualitative differences also exist between the rhizosphere populations of different plants (Gr_2 and Wr_2; Wr_3 and Pr_3) with regard to morphology and the properties according to Gram's staining method.

It is striking that Gram-positive and Gram-variable *Arthrobacters* consistently form a large part of the population of the soil whereas their share in the rhizosphere varies from a minority in the case of young plants in moist soil (Gr_2 and Wr_2), to a relative abundance in the rhizosphere of older plants in a dry soil (Wr_3, Pr_3, and Gr_3). The *Arthrobacters* in the Ws_4 series proved to be largely Gram-negative.

In the rhizosphere of young wheat plants under moist soil conditions a large part (30%) of the population consisted of long flexible Gram-negative rods forming brownish-yellow colonies. In colonies of three weeks old, many cocci were present. This bacterium, which holds stains poorly, seems to be specific for young wheat plants as it was only found here.

Chromogenic isolates. The occurrence of a higher percentage coloured (yellow or orange or brownish-yellow) colonies of bacteria among the isolates from the rhizosphere is already known from literature (Lochhead, 1940; Clark, 1940; King and Wallace, 1956; Rovira, 1956, Sperber and Rovira, 1959; Rivière 1961; Vágnerová, Macura and Catská, 1960; Rangaswami and Vasantharajan, 1962) and was found here for the Wr_2 series (63% chromogenic), but not for Gr_2 which contained a lower percentage (15%) than the Gs_2 series (22%). Probably this phenomenon was also present in the Pr_3 and the Gr_3 series with 47 and 33% chromogenic isolates, respectively. The difference between the Wr_3 and the Ws_3 series, with 26 and 21% chromogenic isolates, respectively, is not significant.

The *generic composition* of the bacterial population of some soil and rhizosphere samples is presented in Appendix II. The percentages have been calculated on the basis of the original number of pure cultures, so including all isolates which died before pure culturing or those lost in the fire (dotted areas).

The percentage of *Arthrobacters* is higher in soil than in the wheat rhizosphere and the percentage of *Arthrobacters* in the rhizosphere population of old wheat plants is higher than in that of young plants. Appendix I (rods becoming cocci) shows that the bacteria isolates of the soil and rhizosphere populations of Bs_2, Ps_2, Gs_2, Gr_2 and Gr_3 contain about 50, 41, 37, 13 and 25% *Arthrobacters*, respectively.

Contrary to *Arthrobacter*, the percentage of *Corynebacters* in the rhizosphere is higher than in the soil. (*Corynebacters* resemble *Arthrobacters* but the often less pleiomorphic rods are never transformed into cocci. Rods become smaller when old but hold their club or wedge shape. As rods of *Arthrobacter*, they show "snapping division", leading to V-forms.)

Brevibacterium occurred in Wr_2 and Ws_2 (1%), in Wr_2 (7%) and Pr_3 (6%). They formed small, short, Gram-positive rods, which kept their rod shape when old. These bacteria are neither *Arthrobacter*, nor *Corynebacter*, do

not decompose cellulose and do not fit into other groups. So *Brevibacterium*, not sharply defined, was left as a possible genus. The type species, *Brevibacterium linens*, however, is more correctly classified as *Arthrobacter* (Mulder and Antheunisse, 1963) and is included in the latter genus in the 8th edition of Bergey's Manual of Determinative Bacteriology, Buchanan and Gibbons (1974).

Mycobacterium representatives were only found in soil (9% in Ws_2 and 2% in Ws_3). They were Gram-positive, slender rods of which a part was acid-fast.

Representatives of the genus *Mycococcus* were present in very low numbers in Ws_2 (4%), Wr_3 (1%), Ws_4 (1%) and Pr_3 (1%). They formed cocci of different sizes. Some of then formed a coccoid rod, somewhat wedge-shaped when very young. Branched forms in young liquid cultures were not observed.

Nocardias with slimy colonies, which resembled bacteria, were rare.

Other *Coryneforms* of which it is not certain that they belonged to one of the genera mentioned were present in Wr_2 and Ws_2 (each 1%), in Ws_3 (3%) and in Pr_3 (5%). A part of them died when pure, so the determination was only tentative.

Micrococcus, Gram-positive, small cocci of uniform size were found in very low numbers in some series (Ws_2, Ws_3 and Pr_3, each 1%, and Wr_3 2%).

Bacillus species were found in Ws_2 (9%), Ws_3 (5%) Wr_2 (1%) and Wr_3 (2%).

Pseudomonas species occurred in all samples, but least in Wr_3 (3%), Ws_4 (5%) and Pr_3 (5%). In other samples, 12-15% of the bacterial population consisted of *Pseudomonads*. (Gram-negative, motile rods with polar flagella, which are oxidase-positive, never ferment glucose in Hugh and Leifson's medium (1953), while many of them desaminate arginine under anaerobic conditions. Pigments are always soluble in water and some of them show fluorescence in U.V. According to the scheme of Hendrie *et al.* (1964), four groups of *Pseudomonads* can be distinguished. Most common were representatives of group II, which oxidize glucose to acid and do not show fluorescence; group I shows fluorescence and was second in importance. Representatives of the groups III and IV did not seem important.

Flavobacterium isolates were found only in the rhizosphere of young wheat plants (10%). On the medium we used, the colonies were orange-yellow; on milk agar, they were bright yellow.

Sporocytophage/Flexibacterium also occurred (16%) only in rhizosphere of young wheat plants. The colonies were brown-yellow, had a typical, unpleasant smell and had around the visible colony a hardly visible brim of 3 - 4 mm, presumably only one or a very few cell layers thick, which when collected had the same colour as the colony. When two days old, 6-14 µm long and slender cells occurred which were sometimes slightly bent. A culture of 2-3 weeks old contained many cocci (0.6-1.4 µm diameter) and short cells. Cells have rounded ends. Growth on cellulose agar was not observed. The generic position is not clear.

One *Agrobacterium* was found in Wr_3 and two in Ws_3: Gram-negative motile cells with one lateral flagellum on one or on each side of the cell. The isolate gave a positive Bernaerts and De Ley (1963) reaction due to the formation of 3-ketoglucosides, and a brown halo on Riker's medium.

Achromobacter was found only once in Ws_4: Gram-negative, non-motile, not pigmented and not giving an alkaline reaction in litmus milk. (This genus is included in the genus *Alcaligenes* in the 8th edition of Bergey's Manual.)

In nearly every sample of soil or rhizosphere, one or a few isolates could not be placed with sufficient accuracy in existing genera.

3.3. Decomposition of different substrates

3.3.1. By the isolates of different samples (mainly from soil and wheat rhizosphere)

The percentages of (pure) isolates of each sample able to decompose a certain substrate are summarized in tabel II. For each sample the average percentage was calculated and presented at the bottom of the table. These values are a measure of the "decomposition potential" of the isolates of a sample. There is a considerable difference between the decomposition potential of Wr_3 (42) and Ws_3 (56).

TABLE II. Percentages of isolates from rhizosphere and soil able to decompose various substrates.

Substrate	Isolates from:						Average
	Wr_2*	Ws_2	Wr_3	Ws_3	Ws_4	Pr_3	
Formate	18	19	35	52	26	39	32 ± 13
Acetate	28	19	39	56	23	63	38 ± 18
Propionate	5	14	9	24	4	18	12 ± 8
Butyrate	10	7	13	31	17	22	17 ± 9
Valerianate	0	2	3	19	0	8	5 ± 7
Oxalate	-	-	6	3	0	6	4 ± 2
Malonate	47	41	35	71	26	59	47 ± 16
Malate	68	80	82	89	67	90	79 ± 10
Citrate	52	57	45	80	74	63	62 ± 13
Benzoate	0	0	4	4	0	6	2 ± 3
Acid from:							
Glucose	100	76	64	79	71	84	79 ± 12
Sucrose	78	56	56	78	74	86	71 ± 13
Lactose	43	41	26	38	38	42	38 ± 6
Xylose	73	63	64	61	38	60	60 ± 12
Mannitol	68	68	61	89	78	86	75 ± 11
Sorbitol	59	46	39	59	45	58	51 ± 9
Hydrolysis of:							
Starch	40	56	52	56	66	92	60 ± 14
Aesculin	65	63	50	64	78	78	66 ± 11
Tween 80 (fat)	43	39	43	52	51	52	47 ± 6
Gelatin	46	61	56	75	74	58	62 ± 11
Ca-Caseinate	38	61	52	64	75	32	54 ± 16
Urea	59	71	58	68	59	54	62 ± 7
Reduction of NO_3 to:							
nitrite	59	73	68	56	66	52	62 ± 8
gas	0	2	5	9	1	8	4 ± 4
H_2S from peptone	59	59	70	86	54	72	67 ± 12
NH_3 from peptone	89	78	92	100	93	100	92 ± 8
NH_3 from arginine (anaerobic)	22	5	10	23	9	10	13 ± 7
Kovacs oxydase test	84	61	48	87	41	60	64 ± 17
"decomposition potential"	46	45	42	56	45	52	48 ± 5
Number of isolates	37	41	84	87	85	50	

* Legends see App. I.

The column on the right gives the average percentage of all isolates able to decompose or to transform a certain substrate. It is a measure of the ease with which a compound can be decomposed. Sometimes clear differences between isolates (populations) from soil and rhizosphere exist.

3.3.2. By the isolates of the different genera

It was shown that the bacterial floras of soil and rhizosphere soil differed in numbers, composition and in the in ability to decompose or transform different compounds.

Table III presents the percentages of isolates from the different genera able to decompose a given compound or a group of compounds.

TABLE III. Percentage of isolates from different genera of bacteria from rhizosphere and soil able to decompose a given substrate or group of substrates.

	All carbohydr. tested	Xylose	Formate	Oxal.	Benzo.	Starch	Protein	Fat (Tween 80)	Gas from nitrate
Arthrobacter (177)	39	63	28	0.5	0.5	79	70	44	2
Corynebacter (94)	30	69	34	2	1	39	24	52	0
Pseudomonas (43)	23	81	37	2	9	28	26	42	21
Bacillus (10)	40	80	30	0	0	90	100	30	0
Mycobacterium (8)	0	38	100	38	0	0	25	50	0

Number of isolates tested in brackets.

Of the 25 substrates tested (table II), the *Arthrobacters* could, on average, decompose 13.8, the *Corynebacters* 11.7 and the *Pseudomonads* 10.9. Within the limits of what was offered as a substrate, the *Arthrobacters* had the best chance to find a useful substrate.

3.4. Sensitivity to some antibiotics

With Oxoid Multodiscs type 30-9B, the sensitivity of each isolate to eight antibiotics was determined on the agar containing soil extract that was always used in this study. The antibiotics in these Multodiscs and their concentrations were: penicillin-G (1.5 unit), chloramphenicol (0,01 mg), erithromycin (0.01 mg), novobiocin (0.005 mg), cloxacillin (0.005 mg), ampicillin (0,002 mg), streptomycin (0,01 mg) and tetracyclin (0.01 mg).

In table IV the percentages of the isolates belonging to the different classes of sensitivity are given.

TABLE IV. Percentage of the isolates of the different genera in different classes of sensitivity to the antibiotics used.

	Very sensitive	Sensitive	Less sensitive	Insensitive	
Arthrobacter	44	44	10	1	(169)
Corynebacter	17	54	21	7	(94)
Mycobacterium	0	0	87	13	(8)
Bacillus	40	50	10	0	(10)
Pseudomonas	5	9	80	7	(43)

Number of isolates tested in brackets.

These classes are: *very sensitive:* sensitive to all antibiotics used, but sensitivity to penicillin or ampicillin or cloxacillin may be less; *sensitive:* less sensitive to penicillin, ampicillin and cloxacillin or insensitive to one of these (usually penicillin), sensitive to the others; *less sensitive:* not sensitive to penicillin, ampicillin and cloxacillin, sensitive to tetracycline and (often) chloramphenicol, varying sensitivity to streptomycin, novobiocin and erythromycin; *insensitive* may mean there is still a (slight) sensitivity to tetracyclin or streptomycin or chloramphenicol or to two of these.

Within the different rhizosphere and soil series of wheat, differences existed with regard to the place the sensitive bacteria occupy in the populations (tabel V). The insensitive group of bacteria was very small in all series. The group of very sensitive ones varied between 11 and 49%. It is peculiar that the two series with these divergent percentages, Ws_3 and Ws_4, had 50% *Arthrobacters* each. This indicates that the *Arthrobacters* were, at least in part, of different strains or even different species.

TABLE V. Percentage of isolates in different series being insensitive and very sensitive to the antibiotics used.

	Wr_2*	Ws_2	Wr_3	Ws_3	Ws_4	Pr_3
Insensitive	5	2	0	8	0	2
Very sensitive	13	31	22	11	49	14

* Legends, see App. I.

4. DISCUSSION

The rhizosphere microflora is a selection from the microflora of the soil, which can be supplemented by the microflora of the seed, especially in the case of seedlings and young plants. The larger and rougher the surface of the seed, bulb or tuber, the larger the contribution of its microflora to the rhizosphere may be.

The rhizosphere microflora consists (mainly) of those species which thrive better than others under the conditions of a soil environment changed by living plant roots. The changes are caused by the activity of the root, i.e. the uptake of oxygen, water and minerals, the production of carbon dioxide, and particularly by the loss of organic matter as volatiles, as solubles ("root exudates"), as sloughed-off cells (calyptra root hairs) and as damaged and dead epidermal and cortex cells. Due to this supply of food the population of microorganisms in the rhizosphere (R) is much more dense than in soil (S) not influenced by living roots. This is very striking for poor soils, where R/S values of 100 and more are common. In a normal, fertile field soil, however, the R/S values are usually lower than 10 (Rouatt and Katznelson, 1961), values which were not always found in our table I.

Soils with different properties have differently composed microbial populations. The pH of the soil is, in this respect, a very important factor. In acid soils, fungi are more numerous than in neutral or slightly alkaline soils and besides that, other species are present in the acid than in the neutral and slightly alkaline environment, although some species are pH-independent within wide limits.

Van Emden (1972) concluded that different crops in the same soil stimulate quite different fungi and that there is even a different after-effect. For the same crop in different soils it appears to hold that the composition of the rhizosphere microflora depends on the composition of the microflora of the soil. Similar conclusions were drawn by Vruggink (1976) concerning the actinomycetes.

In the bacterial flora of the rhizosphere of young wheat plants the group of Gram-negative bacteria predominated (in moist soil); in the

rhizosphere of plants after flowering (in rather dry soil) the group of Gram-positive bacteria formed the majority (App. I, Wr_2 and Wr_3). This profound change in the composition of the rhizosphere microflora is, according to Woldendorp (1978), caused by the fact that the Gram-positive *Arthrobacter* has a much better ability to grow at low food concentrations than the Gram-negative *Pseudomonas*.

The quickly growing *Pseudomonads* in the rhizosphere of young plants had sufficient food and multiplied rapidly till the food became a limiting factor. From then on the *Pseudomonads*, and evidently other Gram-negatives as well, were gradually displaced by the more efficiently growing *Arthrobacters* (and other *Coryneforms*). It is uncertain to what extent a change in food composition leading to higher C/N ratios, was responsible for the increased presence of *Arthrobacter* and relatives. Peterson *et al.* (1965) grew wheat in soils with constant low, normal, and high moisture contents and observed that *Pseudomonads* predominated at low, and *Arthrobacter* at high moisture contents. This is not in agreement with the results of Salonius *et al.* (1970) who used relatively poor gamma-sterilized soil. They observed that decreasing moisture contents favoured *Arthrobacter*. *Pseudomonas* was favoured when the moisture content was high and when glucose was added. Possibly its presence depends more on food than on moisture.

The generic composition of the bacterial flora of rhizosphere and non-rhizosphere soil had not thorougly been studied when we started this project. From the literature it was known that rhizosphere bacteria were predominatly Gram-negative and chromogenic (Lochhead, 1940; Clark, 1940; King and Wallace, 1956; Rovira, 1956; Sperber and Rovira, 1959; Vagnerová, Macura and Catská, 1960; Rivière, 1961; Rangaswami and Vasantharajan, 1962).

A first study about the generic composition of the bacterial flora in rhizosphere and non-rhizosphere soil was made by Vagnerová *et al.* (1960) for wheat. This was followed by Rivière (1960), who only considered the Gram-negative bacteria, and by Papacostea *et al.* (1968). Rouatt *et al.* (1964) studied the bacterial genera in soil and rhizosphere of soya. Loutit, Hillas and Spears (1972) studied the composition of the bacterial flora according to genera in soil and in the rhizosphere of radish under conditions of low and improved molybdenum contents.

Unfortunately, the properties of the soils used (pH, organic matter and organic nitrogen content, clay or silt content) were often not mentioned. The composition of the microflora of a soil depends on the physical and chemical properties of the soil whereby pH is a very important factor. The composition of the bacterial flora (roughly, according to large groups) in a slightly acid sand soil, and a neutral sandy clay loam after a potato crop is given in table VI. When, as shown in table VI, the composition of the bacterial flora of soils can vary so much, it is not surprising that rhizosphere microfloras of the same plant species or variety are different in various soils.

TABLE VI. Composition of the bacterial flora of a pleistocene sand and a holocene marine sandy clay loam. Some properties of the soils are presented.

	Sand		Sandy clay loam	
pH_{KCl}	4.3		7.5	
Org. matter (%)	9.3		6.2	
Clay content (%) (particles <16μ)	6		35	
Gram-negative bacteria (%)				
Pseudomonads	33		12	
Flavobact./Cytophaga	15	59	2	16
Other Gram-negatives	11		2	
Gram-positive bacteria (%)				
Coryneforms	32		50	
Bacillus	1		0	
Nocardia	0	41	28	84
Streptomyces	6		6	
Other Gram-positives	2		0	

The *Nocardias* and the *Streptomycetes* both had a slimy, bacteria-like appearance.

Clark's statement that the principal genus of the predominantly Gram-negative bacteria in the rhizosphere was *Pseudomonas* may be true for the (acid) soil he used. Firsanova (1956) observed that the microflora of roots of rye, grown on a podsolic soil, consisted essentially of bacteria of the genera *Pseudomonas* and *Mycobacterium*. Sperber and Rovira (1959) found among the mainly Gram-negative and often chromogenic types in the rhizosphere of clover and grass 63 and 78% branched forms, respectively *(Arthrobacters* and *Nocardias* and no *Pseudomonads)*. Presumably the soil they used must have had a pH of 7 or higher. It is known that some *Arthrobacters* are Gram-negative when young and in the rod stage (see App. I); *Nocardias* are Gram-positive. This property, however, may vary with age for some species (Bergey's Manual; Buchanan and Gibbons, 1974).

In the rhizosphere of wheat, Rivière (1961) observed representatives of the following Gram-negative genera: *Alcaligenes, Erwinia, Achromobacter, Pseudomonas, Xanthomonas* and *Flavobacterium*. The soil that was not influenced by roots contained a high percentage of bacilli. Peterson and Rouatt (1967) observed that in the rhizosphere of flax, *Pseudomonas* and *Flavobacterium* isolates were the most numerous groups. In the rhizosphere of the *Fusarium*-susceptible cultivar they were more numerous than in the rhizosphere of the resistant cultivar. In the latter, *Arthrobacter, Mycococcus* and *Mycobacterium* were relatively more abundant, although they still formed a minority only. Hollings *et al.* (1969) observed that 62-97% of all bacteria in a New Zealand grassland soil (pH 4.9-6.2) were Gram-negative.

More detailed results of the generic composition of the microflora of soil and rhizosphere of wheat given in table VII have been taken from experiments of Vágnerová *et al.* (1960), Papacoustea *et al.* (1968) and from own experiments; the results of Rouatt *et al.* (1964) for soya are included. Comparing the data of table VII, we can see that specific genera of rhizosphere bacteria (those genera that increase relatively in numbers in the rhizosphere) are for wheat and soya: the genus *Flavobacterium* and further *Pseudomonas* (but not in Flevoland soil). The genus *Corynebacterium* seems to be more numerous in Flevoland soils and *Mycoplana* in the soil used by Vágnerová *et al.* Genera much more numerous in soil than in rhizosphere are *Bacillus, Arthrobacter, Mycococcus* and *Micrococcus*.

TABLE VII. Generic composition of the bacterial flora in the rhizosphere of wheat and soya and in the corresponding soil not influenced by plant roots in soils of different origin.

	I, Wheat		II, Wheat				III, Wheat					IV, Soya	
	young		young		old		young		old		after plowing		
	rhiz.	soil	rhiz.	soil	rhiz.	soil	rhiz.	soil	rhiz.	soil	soil	rhiz.	soil
Achromobacter	5	3											
Agrobacterium							0	0	1	2	0		
Alcaligenes			14	1	3	13							
Arthrobacter	14	7	0	33	0	37	14	28	31	50	50	0	29
Bacillus	10	36	0	27	0	24	1	9	2	5	0	0	35
Brevibacterium	3	5					1	1	7	0	0	32	7
Chromobacterium			5	0	1	1							
Corynebacterium	5	4					13	6	29	8	21		
Coryneforme bact.							1	1	0	3	0		
Flavobacterium	11	5	12	11	39	8	10	0	1	0	0	10	0
Kurthia	1	2											
Micrococcus	8	13					0	1	2	0	1		
Mycobacterium	8	4					0	9	0	2	0	0	5
Myococcus	9	13					0	4	0	0	1		
Mycoplana	11	2											
Nocardia							1	1	0	0	1	0	10
Pseudomonas	14	7	60	10	41	3	14	15	3	12	5	55	0
Sporocytofaga/ Flexibacterium							16	0	0	0	0	3*	13*
Vibrio	1	0											
Not determined			10	18	16	15	1	0	1	2	1		
Dead							26	25	18	12	23		

I: Vagnerová *et al.*, 1960; chernozem soil, plants 3 weeks old when sampled. II: Papacoustea *et al.*, 1968; forest podsol, plants sampled in March and May (old). III: This study; sandy loam, pH 7.6, sampled in May and July (old). IV: Rouatt *et al.*, 1964; no further details given. * *Cytophaga*.

Alcaligenes spp. and *Sporocytophaga/Flexibacter* spp. were more numerous in the rhizosphere of young plants than in soil; each of these only occurred in one soil.

The soils of table VII were all different. As a result, large differences in the bacterial flora of the soil and of the plant's rhizosphere occurred. Furthermore the plants were harvested at different growth stages; this influenced the composition of the bacterial flora. We should also keep in mind that the wheat in table VII belonged to different varieties and was grown under different weather conditions; this probably also influenced the composition of the bacterial floras (Neal *et al.*, 1973).

Notwithstanding all causes leading to differences in the composition of the bacterial flora of the rhizosphere, typical rhizosphere bacteria exist. They are representatives of the genera *Flavobacterium, Pseudomonas, Mycoplana, Alcaligenes, Corynebacterium, Brevibacterium, Sporocyrophaga/*

Flexibacterium and possibly also others *(Arthrobacter)*. The proportion of each genus in the population of the rhizosphere strongly depends on soil properties such as pH, on plant species or variety, on growth stage and possibly also on climate and weather conditions.

5. SUMMARY

The aerobic bacteria from the rhizosphere of young and old plants, and from the corresponding soil not influenced by roots, were studied on a crop rotation plot in the polder Oostelijk Flevoland where wheat, rye grass, sugar beet and potatoes were grown for six years in succession.

The following properties of bacteria isolated from soil and rhizosphere were compared: morphology, reaction to Gram's stain, chromogenesis, the genus to which the isolate belongs, the ability to decompose or transform 25 different substrates, and sensitivity to eight antibiotics.

It was found that the isolates from the rhizosphere of young and old plants differed from those of the corresponding soil and that they were also mutually different. *Arthrobacter* was the most important genus isolated from soil. *Arthrobacter* and *Corynebacter* were present in about equal amounts in the isolates from young wheat plants (together 25%), but they were much more numerous (again in about equal proportions) in the rhizosphere of older wheat plants (together 60%). The genera *Flavobacterium* and *Sporocytophaga/Flexibacterium* were specific for the rhizosphere of young wheat plants. *Pseudomonads* were present, but could not be regarded as specific. *Corynebacterium* can be regarded as specific for the rhizosphere of wheat, especially for older plants. Respresentatives of this genus were also rather abundant in the rhizosphere of older potato plants. After plowing, they again were present in rather large numbers in the soil on which wheat had been grown.

6. LITERATURE

Bernaerts, M.J. and J. de Ley, 1963. A biochemical test for crown gall bacteria. Nature 197: 406-407.

Breed, R.S., E.G.D. Murray and N.R. Smith, 1957. Bergey's manual of determinative bacteriology, 7th edition. The Williams and Wilkins Comp., Baltimore, 1094 pp.

Buchanan, R.E. and N.E. Gibbons, 1974. Bergey's manual of determinative bacteriology, 8th edition. The Williams and Wilkins Comp., Baltimore, 1246 pp.

Clark, F.E., 1940. Notes on types of bacteria associated with plant roots. Kansas Acad. Sci. Trans. 43: 75-85.

Emden, J.H. van, 1972. Soil mycoflora in relation to some crop-plants. EPPO Bull. 7: 17-26.

Firsanova, A.N., 1956. The microflora of the rhizosphere (radical area) of rye in relation to development. Zap Leningradsk Sel'dkokhoz Inst. 11, 92-99. (Biol. Abstr. 36, 95688, 1965).

Hendrie, M.S., W. Hodgkiss and J.M. Shewan, 1964. Considerations on organisms of the achromobacter - alcaligenes group. Ann. Inst. Pasteur Lille 15: 43-59.

Hiltner, R., 1904. Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb. DLG 98: 59-79.

Hollings, P.E., M.E. Dutch and J.D. Stout, 1969. Bacteria of four Tussock grassland soils on the Old Man Range, Central Otago, New Zealand. N.Z. J. Agric. Res. 12: 177-192.

Hugh, R. and E. Leifson, 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J. Bact. 66: 24-26.

Kaunat, H., 1961. Wuchsstoffbedürftige Bakterien aus der engen Rhizosphäre von Kulturpflanzen. Zentralbl. Bakteriol. etc. Abt. 2, 114: 233-240.

Kaunat, H., 1963. Zum Problem der Spezifität der Rhizosphärenmikroflora von Kulturpflanzen. III. Wirkung der engen Rhizosphäre auf das Artenspektrum der aeroben nicht-sporenbildenden Bakterien. Zentralbl. Bakteriol. etc., Abt. 2, 117: 283-305.

Lochhead, A.G., 1940. Qualitative studies of soil microorganisms. III. Influence of plant growth on the character of the bacterial flora. Can. J. Res. Sect. C. 18: 42-53.

Lochhead, A.C. and R.H. Thexton, 1947. Qualitative studies of soil microorganisms VII. The "rhizosphere effect" in relation to the amino acid nutrition of bacteria. Can. J. Res. Sect. C. 25: 20-26.

Loutit, M., J. Hillas and G.F.S. Spears, 1972. Studies on rhizosphere organisms and molybdenum concentration in plants I. Identification of rhizosphere isolates to generic level. Soil Biol. Biochem. 4: 261-265 and II. Comparison of isolates from the rhizosphere of plants grown in two soils under the same conditions. Soil. Biol. Biochem. 4: 267-270.

Macura, J. and V. Vančura (Eds.), 1965. Plant Microbes Relationships. Publishing House of the Czechoslovak Academy of Sciences.

Mulder, E.G. et J. Antheunisse, 1963. Morphologie, physiologie et écologie des Arthrobacter. Ann. Inst. Pasteur Paris 105: 46-74.

Neal, J.L., Ruby I. Larson and T.G. Atkinson, 1973. Changes in rhizosphere populations of selected physiological groups of bacteria related to substitution of specific pairs of chromosomes in spring wheat. Plant Soil 39: 209-211.

Papacostea, P., E. Missirlice and E. Popa, 1968. Studiul comparativ al florei bacteriene din rizosfera griului si a unor plante apartinid vegetatiei spontane. Microbiologia (Bucharest) 1: 503-507.

Peterson, E.A., J.W. Rouatt and H. Katznetson, 1965. Microorganisms in the root zone in relation to soil moisture. Can. J. Microbiol. 11: 483-489.

Peterson, E.A. and J.W. Rouatt, 1967. Soil microorganisms associated with flax roots. Can. J. Microbiol. 13: 199-203.

Rangaswami, G. and V.N. Vasantharajan, 1962. Studies on the rhizosphere of citrus trees II. Qualitative distribution of the bacterial flora. Can. J. Microbiol. 8: 479-484.

Rivière, J., 1960. Étude de la rhizosphère du blé. Ann. Agron. 11: 397-440.

Rivière, J., 1961. Action des microorganismes de la rhizosphère sur la croissance de blé I. Répartition des genres et des groupes nutritionels. Ann. Inst. Pasteur 101: 611-618.

Rivière, J., 1963. Rhizosphère et croissance du blé. Ann. Agron. 14: 619-653.

Rouatt, J.W. and H. Katznelson, 1961. A study of the bacteria on the root surface and in the rhizosphere soil of crop plants. J. Appl. Bacteriol. 24: 164-171.

Rouatt, J.W., H. Katznelson and G. Eger, 1964. Observations on the use of different media to evaluate the rhizosphere effect. Proc. 8th Intern. Congress of Soil Science, Bucharest, Vol. III: 609-615.

Rovira, A.D., 1956. A study of the development of the root surface microflora during the initial stages of plant growth. J. Appl. Bacteriol. 19: 72-79.

Rovira, A.D., 1965. Interactions between plant roots and soil microorganisms. Annu. Rev. Microbiol. 19: 241-266.

Salonius, P.O., J.B. Robinson and F.E. Chase, 1970. The mutual growth of Arthrobacter globiformis and Pseudomonas fluorescens in gamma-sterilized soil. Plant Soil 32: 316-326.

Skerman, V.B.D., 1959. A guide to the identification of the genera of bacteria. The Williams and Wilkins Comp., Baltimore, 217 pp.

Sperber, J.I. and A.D. Rovira, 1959. Study of the bacteria associated with the roots of subterranean clover and Wimmera ryegrass. J. Appl. Bacteriol. 22: 85-92.

Starkey, R.L., 1929[a]. Some influences of the development of higher plants upon the microorganisms in the soil: I. Historical and introductory. Soil Sci. 27: 319-335.

Starkey, R.L., 1929[b]. Some influences of the development of higher plants upon the microorganisms in the soil: II. Influence of the stage of plant growth upon abundance of organisms. Soil Sci. 27: 355-379.

Starkey, R.L., 1958. Interrelations between microorganisms and plant roots in the rhizosphere. Bacteriol. Rev. 22: 154-172.

Vágnerová, K., J. Macura and V. Čatská, 1960. Rhizosphere microflora of wheat. I. Composition and properties of bacterial flora during the first stages of wheat growth. Folia Microbiol. 5: 298-310.

Velvis, H., 1975. De samenstelling van de bacteriepopulatie van de rhizosfeer van enkele landbouwgewassen en van niet-doorwortelde grond. Inst. Bodemvruchtbaarheid, Nota 21, 24 pp.

Vruggink, H., 1976. Influence of agricultural crops on the actinomycetes flora in soil. Plant Soil 44: 639-654.

Wallace, R.H. and A.G. Lochhead, 1949. Qualitative studies of soil microorganisms: VIII. Influence of various crop plants on the nutritional groups of soil bacteria. Soil Sci. 67: 63-69.

Wallace, R.H. and H.L. de King, 1954. Nutritional groups of soil bacteria on the roots of barley and oats. Soil Sci. Soc. Am. Proc. 18: 282-285.

Woldendorp, J.W., 1978. The rhizosphere as part of the plant-soil system. In: Structure and functioning of plant populations. Verh. K. Acad. Wet. Pudoc, Wageningen.

APPENDICES

APPENDIX I. COMPOSITION OF THE BACTERIAL FLORA OF SOIL AND RHIZOSPHERE ACCORDING TO GRAM'S STAIN AND MORPHOLOGY

Central circle:

 G- Gram-negative bacteria

 G+ Gram-positive bacteria

 G± Gram-variable bacteria

In the central circle also information about the plots is presented. The capital letter stands for the plots (G= grass plot, P= potato plot, B= beet plot, W= wheat plot); the second letter stands for soil (s) or rhizosphere (r); the figure stands for the sampling date (2= 2nd sampling, 3= 3rd sampling, 4= 4th sampling).

Morphological groups within each Gram-group (in percentages):

 small rods

 rods

 long rods

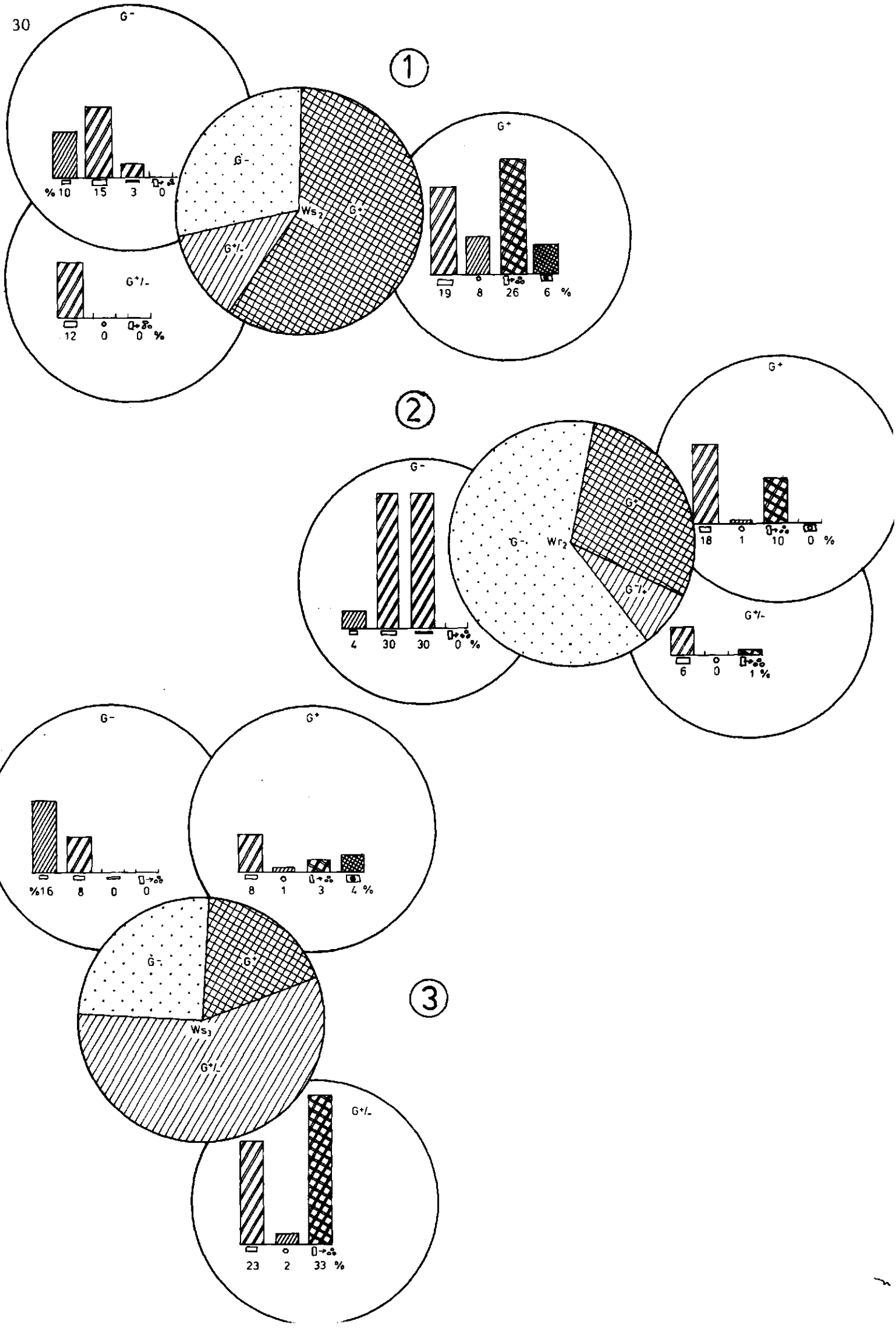
 rods that form cocci (Arthrobacter)

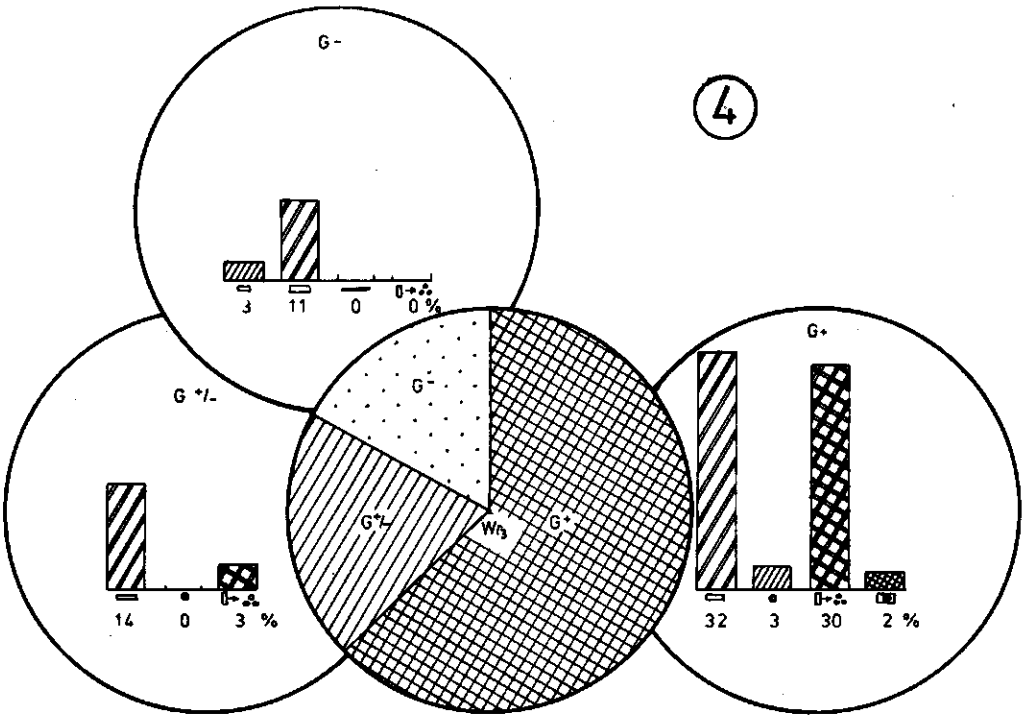
 spore-forming rods (Bacilli)

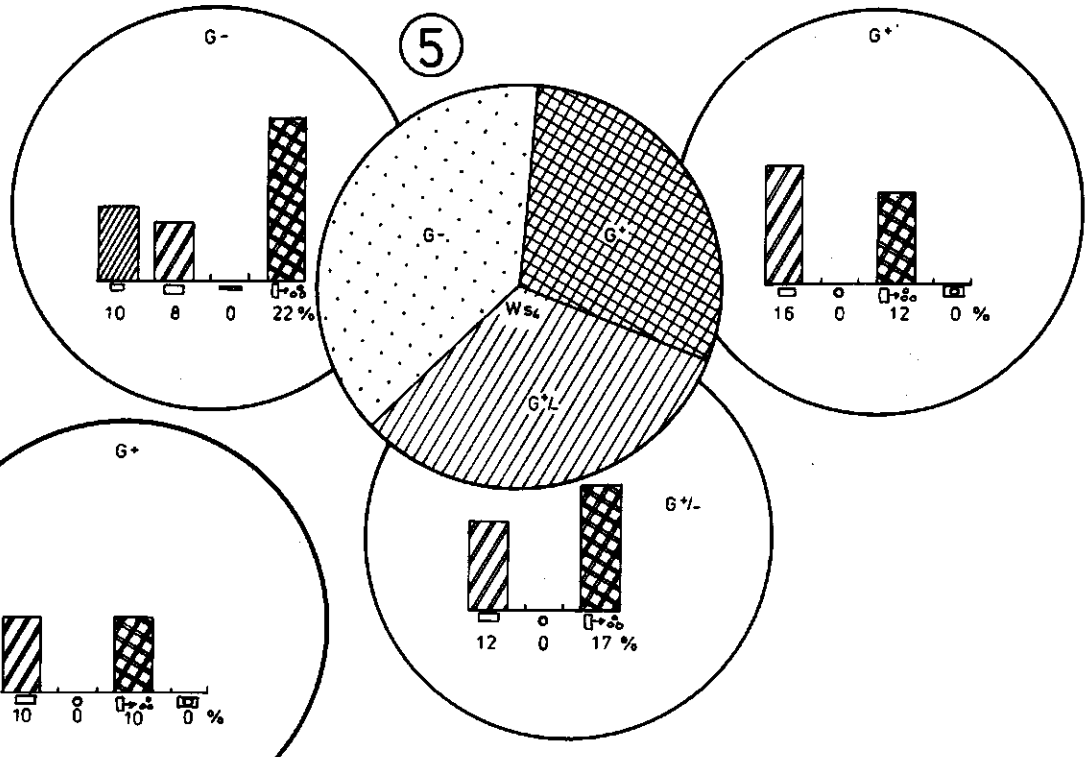
 cocci

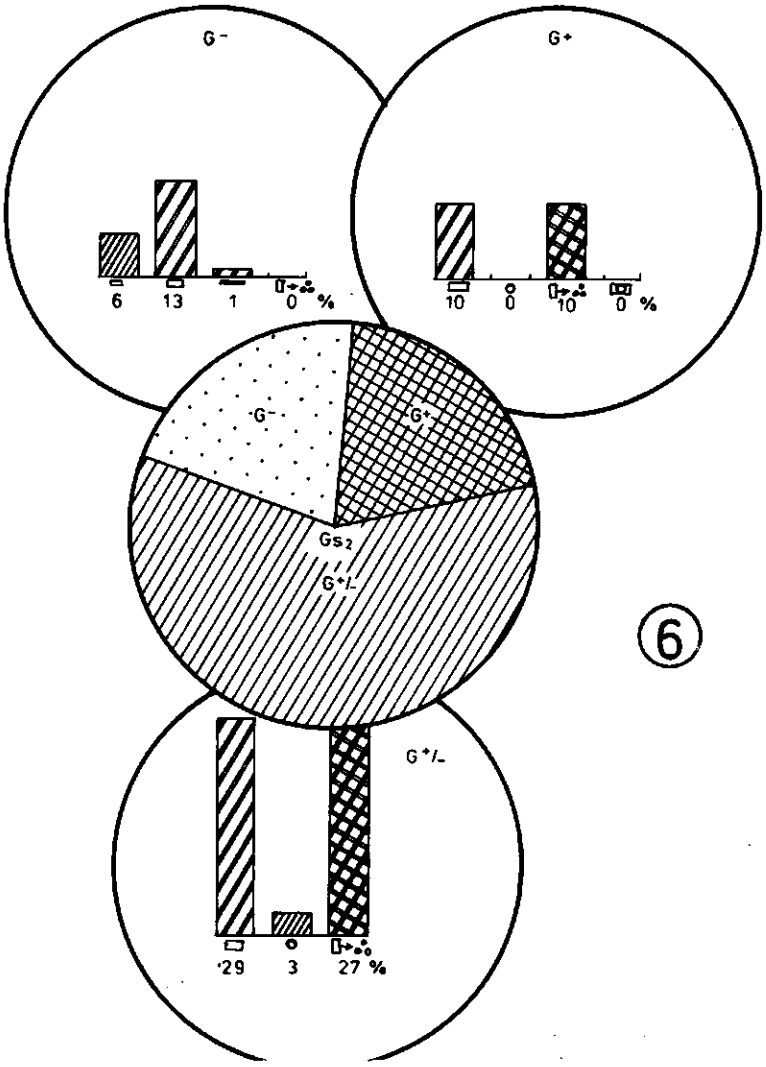
The first five figures show bacterial floras of wheat rhizosphere and soil at different stages of growth.

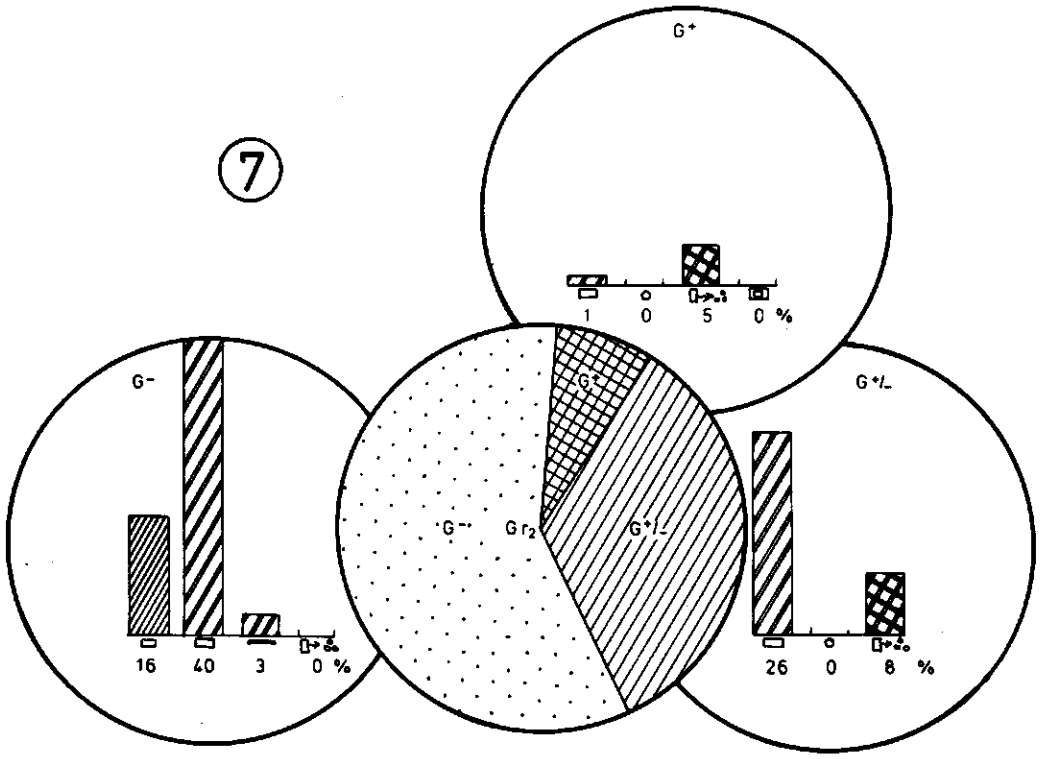
Figures 6-11 show bacterial floras of grass rhizosphere (Gr) and soil (Gs), beet soil (Bs) and potato rhizosphere (Pr) and soil (Ps).

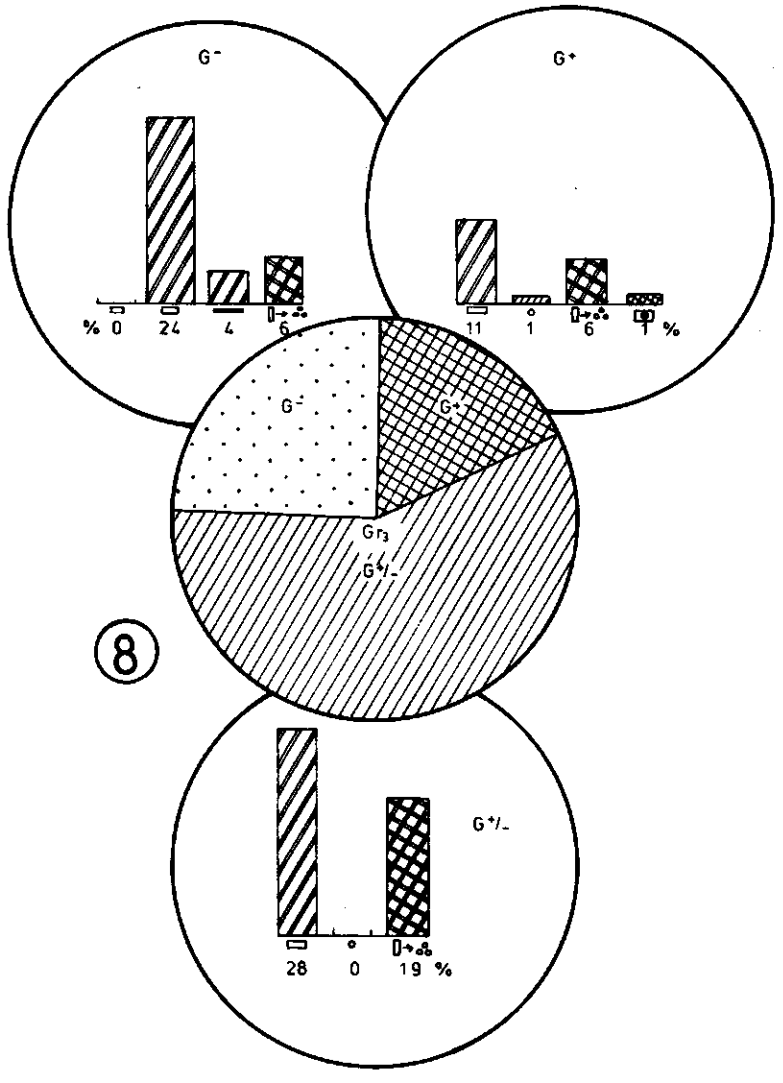

1
G⁻
%10 15 3 0
G⁻
Ws₂
G⁺/₋
G⁺
19 8 26 6 %
G⁺/₋
12 0 0 %
2
G⁻
4 30 30 0 %
G⁻
Wr₂
G⁺
G⁺/₋
G⁺
18 1 10 0 %
G⁺/₋
6 0 1 %
G⁻
%16 8 0 0
G⁺
8 1 3 4 %
3
G⁻
G⁺
Ws₃
G⁺/₋
G⁺/₋
23 2 33 %

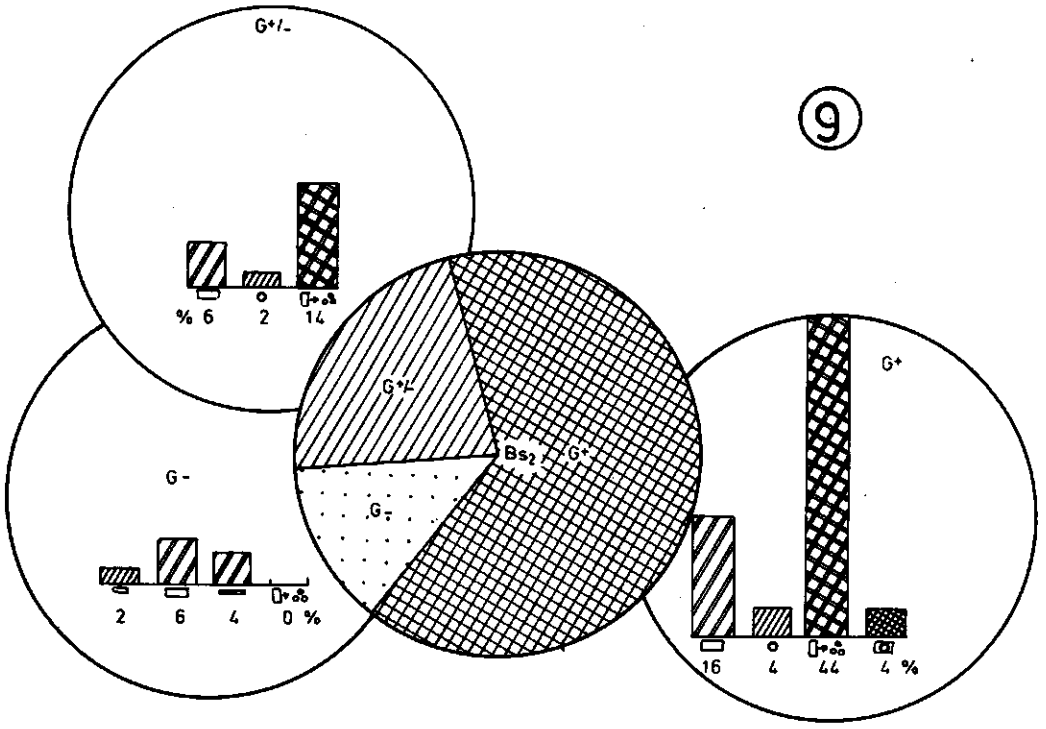

4
G-
3 11 0 0 %
G+/-
14 0 3 %
G-
G+/-
Ws3
G+
G+
32 3 30 2 %

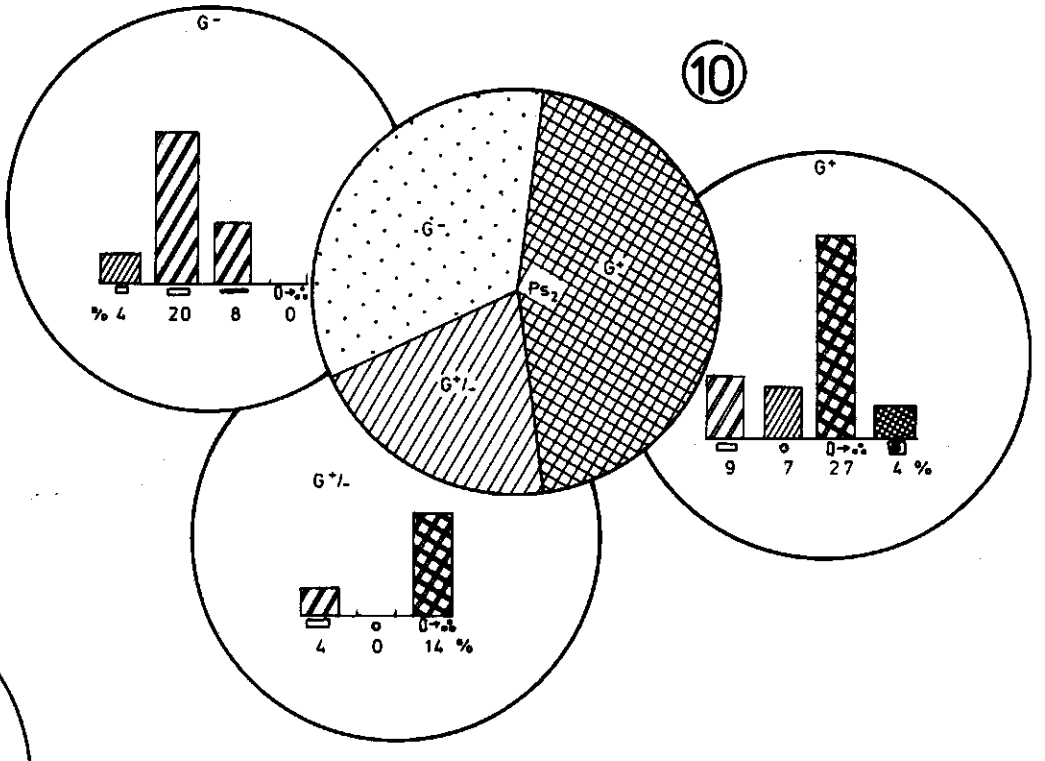

5
G-
10 8 0 22 %
G-
G+
Ws4
G+/-
G+
16 0 12 0 %
G+/-
12 0 17 %

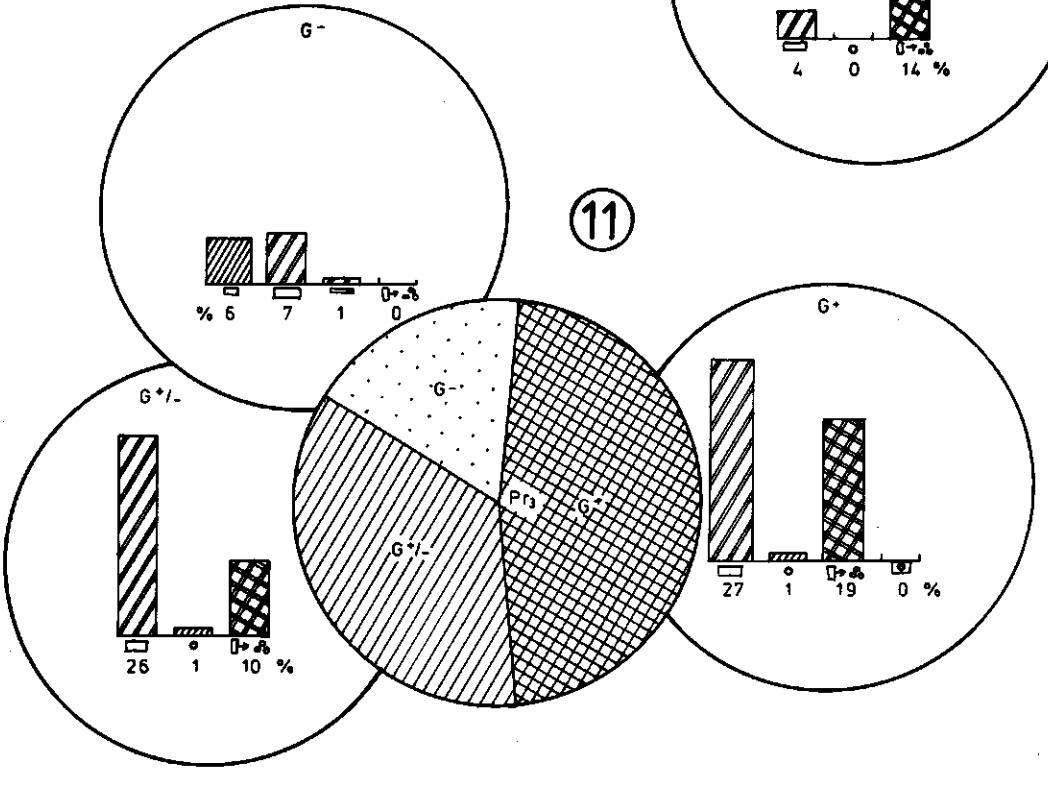

G-
6 13 1 0 %
G+
10 0 10 0 %
G-
G+
Gs2
G+/-
6
G+/-
29 3 27 %

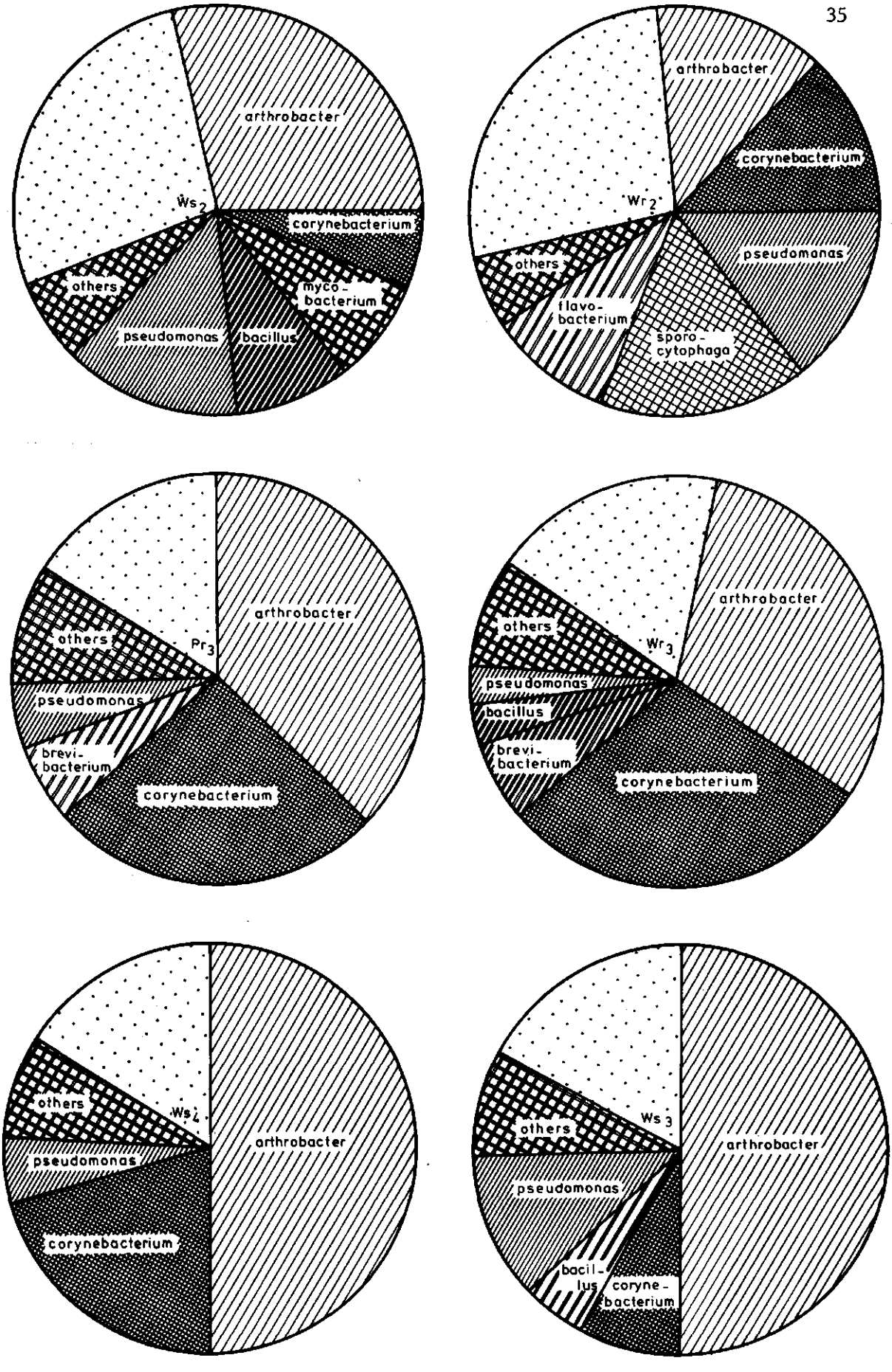

⑦
G+
1 0 5 0 %
G-
16 40 3 0 %
G+/-
26 0 8 %
G--
Gr2
G+/-


⑧
G-
% 0 24 4 6
G+
11 1 6 1 %
G-
G+
Gr3
G+/-
G+/-
28 0 19 %


9
G+/-
% 6 2 14
G-
2 6 4 0 %
G+/-
Bs2
G+
G-
G+
16 4 44 4 %


10
G-
% 4 20 8 0
G-
G+
Ps2
G+/-
G+
9 7 27 4 %
G+/-
4 0 14 %


11
G-
% 6 7 1 0
G+/-
26 1 10 %
G-
Pr3
G+
G+/-
G+
27 1 19 0 %



Appendix II. The generic composition of the bacterial floras of wheat soil (Ws) and wheat rhizosphere (Wr) at different growth stages and of the rhizosphere of potatoes (Pr).