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Rhizosphere Microflora of Wheat

I. Composition and Properties of Bacterial Flora during the First Stages of Wheat Growth

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The soil microflora is exposed to the influence of numerous factors, higher plants playing the most marked role in its composition and properties. Through the action of roots during plant growth quite specific conditions for the growth of microorganisms are formed in the root environs. From the point of view of microbial activity, an important environment is found in the zone of root influence, the environment differing from that outside direct root influence. On the basis of numerous data of the literature the conclusion can be drawn that the microflora in the root system zone differs quantitatively and qualitatively from the microflora of control soil. The greatest number of microorganisms can be found on the roots and in their immediate vicinity, the number decreasing with increasing distance from the roots. Katznelson (1946) introduced the term "rhizosphere effect" which expresses the coefficient of bacterial incidence in rhizosphere as compared with control soil.

Although the rhizosphere effect has been confirmed by numerous findings its significance is not yet quite clear (Starkey, 1958). It remains an open question whether it is possible to control the rhizosphere population. For this it would be necessary to obtain detailed information on the factors controlling microbial development in the rhizosphere and on the properties of the rhizosphere population, on mutual relations of its components as well as on their physiology and ecology. For this reason much attention is being devoted to the study of the bacterial flora of the rhizosphere.

Quantitative and qualitative differences between the bacterial flora of the rhizosphere and that of the soil have been clearly demonstrated (Clark, 1949; Katznelson, Lochhead & Timonin, 1948; Krasil'nikov, 1958; Starkey, 1958). Rhizosphere bacteria differ from those of the soil in their nutritional requirements. They are characterized by an increased incidence of bacteria requiring amino acids (Lochhead & Thexton, 1947; Lochhead & Rouatt, 1955). Bacteria isolated from the rhizosphere are physiologically more active (Lochhead, 1940; Katznelson & Rouatt, 1957) and they grow more rapidly than soil bacteria (Rovira, 1956; Rouatt & Katznelson, 1957). Rouatt and Katznelson (1957) therefore expressed the assumption that bacteria which are more active physiologically and from the point of view of growth will predominate in the rhizosphere. Macura (1958) showed that on wheat roots, soil bacteria with more profuse growth predominate over the bacteria of seeds. The rhizosphere types also display higher metabolic activity and greater rate of oxygen consumption with glucose and alanine as substrates than soil bacteria (Zagallo & Katznelson, 1957).

It can be assumed that in addition to the rhizosphere zone conditions, properties of the bacteria themselves influence the colonization of the rhizosphere zone by various microorganisms. In the present paper an attempt is described to investigate in more detail the bacterial

flora of the root surface, of the rhizosphere and of control soil during the initial stages of wheat growth with the aim of establishing the differences in the composition and properties of the bacterial population of these three zones in a period when the rhizosphere effect begins to show. Emphasis is placed on the role of seed bacteria, on the one hand, and of soil bacteria, on the other, in the composition of the rhizosphere population.

MATERIALS AND METHODS

Cultivation of wheat. The bacterial flora of the rhizosphere was investigated during the first stages of wheat growth. The wheat sort Česká přesívka (Bohemian alternative) was used, being grown in a greenhouse in pots containing 1.5 kg. soil (chernozem soil from Vinoř). Soil humidity was kept at 60% water holding capacity for the whole duration of the experiment. Samples for microbiological analyses were removed 7, 14 and 21 days after sowing. Each lot contained samples from 4 pots which were treated separately. Prior to the experiment an analysis of bacterial flora from the seed surface and from the soil used was carried out.

Removal and treatment of samples. The samples were analysed on the day of removal. The number of bacteria on root surface, in rhizosphere soil and in control soil was determined. A fallow soil from unsown pots served as control. The term rhizosphere soil is taken to denote the soil which adhered to the roots after shaking and could be removed only mechanically. For analysis of root flora roots were used from which rhizosphere soil had been removed and which were washed in sterile water. For the preparation of the basic suspension the roots or the soil sample were mixed for 2 min. in a Waring Blendor. One ml. suspension of various dilutions was pipetted into sterile Petri dishes and mixed with molten agar medium.

Qualitative characteristic of bacterial flora. For the determination of bacterial count and for their isolation Taylor's medium was employed (1951). Bacterial count was taken after 10 days of incubation at 28° C. From dishes at suitable dilutions, all colonies either from the whole dish or from a certain sector were isolated by the non-selective method of Lochhead and Chase (1943) and transferred by inoculation to a semi-solid medium of the same composition as that used for isolation but containing 0.3% agar. During each repetition 60 colonies were isolated, so that from one zone during a single removal 240 cultures were obtained. The classification of isolated cultures into morphological groups was carried out on the basis of microscopic examination of preparations stained by Gram.

Nutritional requirements were determined by a somewhat modified method of Lochhead and Chase (1943). Three media were used in this connection: Medium B contained 0.05% $(NH_4)_2HPO_4$ and 0.1% glucose, in addition to mineral salts, medium A contained in addition 0.4% Casamino Acids Difco, medium YS contained 0.3% Michrome yeast extract and 25% soil extract.

After ten days of incubation at 28° C the growth of isolates in all the three media was estimated by measuring transmission on a photoelectric colorimeter. The values obtained were used for the determination of intensity of growth of bacteria isolated from various zones as well as of their nutritional requirements. Significant growth (i.e. $< 80\%$ transmission) served as criterion for placing the culture into one of the three nutritional groups.

Common biochemical tests were used for the identification of the bacteria as may be found in the Manual of Microbiological Methods (1957). Identification of the genera isolated was carried out according to Bergey's Manual of Determinative Bacteriology (1957).

RESULTS

The determination of the number of bacteria on roots, in the rhizosphere soil

Table 1. Number of bacteria on root surface, in the rhizosphere and control soil during the first stages of growth of wheat and moisture content. Numbers are given in millions per g. fresh weight of roots or per g. soil of natural humidity.

Zone	Days after planting 7	14	21
	Number of bacteria		
Root surface	659.3 ± 70.75	545.9 ± 29.34	2629.6 ± 531.65
Rhizosphere soil	60.2 ± 8.10	74.4 ± 6.86	100.5 ± 12.86
Control soil	45.0 ± 1.30	37.5 ± 1.50	42.8 ± 4.67
	Moisture content in %		
Rhizosphere soil	23.4 ± 0.55	22.5 ± 0.16	20.5 ± 0.95
Control soil	27.7 ± 0.43	22.8 ± 0.30	23.8 ± 0.58

and in control soil provided evidence that the rhizosphere effect is displayed by wheat in numbers of bacteria as early as in the stage of seedlings (Table 1). An analysis of the properties of bacteria isolated from the above three zones showed that the rhizosphere effect is displayed even qualitatively. In wheat seedlings, there is a predominance of Gram-negative bacterial forms on the root surface and in the rhizosphere soil from the very first days of growth (Table 2). In control soil, on the other hand, Gram-positive organisms represent a considerable part of bacterial flora. During the first three weeks of growth wheat roots are found to possess a greater number of Gram-negative rods and a smaller number of Gram-negative short and coccoid rods. In rhizosphere soil, both forms of Gram-negative bacteria are found to decrease in number. On the other hand, in control soil, the incidence of Gram-negative and Gram-variable organisms increases. During the first stages of growth, the roots and the rhizosphere soil were also observed to possess an increasing number of long non-sporulating and sporulating Gram-positive bacteria.

Marked differences in the occurrence of various morphological types of bacteria were observed during the analysis of bacterial flora of seeds and of soil used in the experiments. Gram-negative forms were clearly predominant on seeds, Gram-negative rods being particularly abundant. In non-wetted soil, before sowing the seeds, the greatest relative incidence of Gram-positive cocci and Gram-negative rods was found while sporulating microorganisms were only sparsely represented. After wetting the soil, the ratio between the individual morphological groups of bacteria changed in that in the unsown soil kept at constant humidity a marked rise in the number of sporulating bacteria and a decrease in the number of Gram-positive cocci and Gram-negative rods could be observed.

In comparison with the bacterial flora of seeds the roots are found to contain more abundant Gram-positive bacteria, both cocci and non-sporulating and sporulating rods, while Gram-negative organisms are less frequently represented.

Bacterial flora of root surface, of rhizosphere and control soil differ markedly in the occurrence of bacteria with various nutritional requirements (Table 3). Average values from all removals show that on the roots all the three groups of bacteria with different nutritional requirements are represented in approximately identical amounts. In the direction from the roots to the soil the percentage of bacteria requiring amino acids decreases.

Table 2. Relative incidence of various morphological groups of bacteria on seeds, root surface, in rhizosphere and control soil during the first stages of growth of wheat. The values are expressed as per cent of total number of isolates.

Days after sowing	Gram-negative bacteria			Total
	Short coccoid rods	Rods	Gram-variable	
		Seeds		
	29.5	49.1	8.2	86.8
		Soil before planting		
	22.2	17.8	11.1	51.1
		Root surface		
7	38.8	27.7	8.2	74.7
14	25.2	30.5	5.3	61.0
21	20.7	33.3	8.1	62.1
Average	29.9	29.9	7.1	66.9
		Rhizosphere soil		
7	26.3	29.6	16.7	72.6
14	24.8	26.2	2.1	53.1
21	19.2	23.4	10.6	53.2
Average	23.6	26.3	8.7	58.6
		Control soil		
7	15.3	10.4	6.8	32.5
14	14.4	15.0	7.8	37.2
21	16.3	18.5	11.9	46.7
Average	15.3	14.4	8.6	38.3
	Gram-positive bacteria			Total
	Cocci	Non-sporulating rods	Sporulating bacteria	
		Seeds		
	5.0	5.0	3.2	13.2
		Soil before planting		
	37.8	6.7	4.4	48.9
		Root surface		
7	15.9	4.7	4.7	25.3
14	22.5	9.9	6.6	39.0
21	17.2	10.4	10.3	37.9
Average	18.6	7.9	6.6	33.1
		Rhizosphere soil		
7	17.9	6.3	3.2	27.4
14	31.7	9.0	6.2	46.9
21	12.8	17.0	17.0	46.8
Average	22.5	10.5	8.4	41.4
		Control soil		
7	17.2	12.3	38.0	67.5
14	13.4	7.2	42.2	62.8
21	25.2	8.1	20.0	53.3
Average	18.0	9.2	34.5	61.7

Table 3. Relative incidence of bacteria with various nutritional requirements, on seeds, on root surface, in rhizosphere and control soil during the first stages of growth of wheat. The values are expressed in per cent of total number of isolates.

Days after sowing	Nutritional groups		
	B	A	YS
		Seeds	
	12.6	44.0	43.4
		Soil before planting	
	48.6	25.7	25.7
		Root surface	
7	32.3	40.3	27.4
14	28.7	31.1	40.2
21	39.3	31.0	29.7
Average	33.3	34.4	32.3
		Rhizosphere soil	
7	34.4	26.0	39.6
14	35.3	30.0	34.7
21	28.9	31.7	39.4
Average	33.0	29.1	37.9
		Control soil	
7	44.8	25.1	30.1
14	40.9	27.3	31.8
21	35.9	24.1	40.0
Average	40.7	25.6	33.7

Whereas in the rhizosphere soil the highest incidence of bacteria with complex nutritional requirements was observed, in control soil bacteria with simple nutritional requirements are more abundant. In contrast to soil analysed before the experiment, wetted control soil contains less bacteria growing in a medium with mineral nitrogen and more bacteria requiring substances contained in the yeast and soil extracts.

Even in the case of the occurrence of nutritional groups of bacteria there was a marked shift in the composition of bacterial flora of the root surface as compared with bacterial flora of seed surface. On the roots the occurrence of bacteria with simple nutritional requirements increased while the occurrence of the other two groups of bacteria was lowered.

On comparing the nutritional requirements of various morphological groups of bacteria it was shown that there are differences in nutritional requirements not only between various morphological groups of bacteria but also within a single morphological group, depending on the zone from which the corresponding bacteria have been isolated (Table 4). For bacteria isolated from the seed surface, a high incidence of bacteria with complex nutritional requirements (with the exception of Gram-variable rods) and a low incidence of bacteria with simple nutritional requirements are characteristic. Among the Gram-negative bacteria isolated from the soil before the experiment the complex-

Table 4. Nutritional requirements of morphological groups of bacteria.

Origin	Nutritional groups B	A	YS
	Gram-negative short coccoid rods		
Seeds	16.3	39.5	44.2
Soil before planting	22.2	22.2	55.6
Root surface	35.0	40.0	25.0
Rhizosphere soil	34.4	28.1	37.5
Control soil	50.0	25.8	24.2
Average	39.1	32.6	28.3
	Gram-negative long rods		
Seeds	1.5	39.4	59.1
Soil before planting	22.2	22.2	55.6
Root surface	32.3	31.3	36.4
Rhizosphere soil	27.9	41.2	30.9
Control soil	29.3	29.3	41.4
Average	30.2	33.8	36.0
	Gram-variable rods		
Seeds	31.2	37.5	31.3
Soil before planting	20.0	40.0	40.0
Root surface	54.2	16.6	29.2
Rhizosphere soil	31.8	13.6	54.6
Control soil	57.9	18.4	23.7
Average	50.0	16.7	33.3
	Gram-positive cocci		
Seeds	0.0	28.6	71.4
Soil before planting	47.1	17.6	35.3
Root surface	40.0	17.1	42.9
Rhizosphere soil	35.0	18.3	46.7
Control soil	43.1	21.5	35.4
Average	39.7	19.1	41.2
	Gram-positive non-sporulating rods		
Seeds	12.5	25.0	62.5
Soil before planting	66.7	33.3	0.0
Root surface	20.0	12.0	68.0
Rhizosphere soil	30.0	20.0	50.0
Control soil	26.3	28.9	44.8
Average	25.8	21.5	52.7
	Sporulating bacteria		
Seeds	0.0	20.0	80.0
Soil before planting	50.0	50.0	0.0
Root surface	19.0	52.4	28.6
Rhizosphere soil	34.6	30.8	34.6
Control soil	36.6	29.2	34.2
Average	34.6	31.7	33.7

nutrition group is most abundant, while among Gram-positive bacteria the simple-nutrition group predominates. In bacteria isolated from the root surface, from the rhizosphere and control soil during the first stages of wheat growth, nutritional requirements of bacteria of the same morphological type will frequently differ.

As shown in Table 5 the individual nutritional groups of bacteria are represented by different morphological types. The ratio of individual morphological types in the composition of nutritional groups is associated with the source of the corresponding bacterial flora. The group of bacteria with simple nutritional requirements was represented in the soil before the experiment first of all by Gram-positive cocci, in control soil during the first stages of wheat growth by Gram-positive sporulating microorganisms, cocci and Gram-negative minute rods, on the root surface and in the rhizosphere soil mostly by Gram-negative microorganisms and by Gram-positive cocci. In the group of bacteria requiring amino acids, Gram-negative bacteria predominate in the seed bacterial flora, in the soil before planting Gram-positive cocci, Gram-negative and Gram-variable organisms are most abundant. In control soil, within this nutritional group, there is a marked rise in the incidence of Gram-positive organisms during the first three weeks of wheat growth, while the incidence of other types decreases. Among the bacteria of this nutritional group isolated from the root surface and from rhizosphere soil, Gram-negative organisms predominate. In the group of bacteria with complex nutritional requirements Gram-negative rods and short coccoid rods are most abundant among the organisms isolated from the seeds, while Gram-variable and both types of Gram-negative bacteria predominate among bacteria isolated from soil. The third nutritional group is represented on the roots by Gram-negative and Gram-variable forms, in the rhizosphere soil and even more markedly

Table 5. Ratio of individual morphological types of bacteria in nutritional groups.

Nutritional groups	G−coccoid rods	G−rods	G−variable	G+cocci	G+nonsporulating	G+sporulating
			Seeds			
B	50.0	7.2	35.7	0.0	7.1	0.0
A	31.5	48.1	11.1	3.7	3.7	1.9
YS	24.7	50.6	6.5	6.5	6.5	5.2
			Soil before planting			
B	12.5	12.5	6.3	50.0	12.5	6.2
A	18.2	18.2	18.2	27.2	9.1	9.1
YS	27.8	27.8	11.1	33.3	0.0	0.0
			Root surface			
B	29.9	27.4	11.1	23.9	4.3	3.4
A	39.6	30.7	4.0	11.9	3.0	10.8
YS	20.7	29.7	5.8	24.8	14.1	4.9
			Rhizosphere soil			
B	25.3	21.8	8.0	24.1	10.4	10.4
A	24.3	37.8	4.0	14.9	8.2	10.8
YS	22.0	19.3	11.0	25.7	13.8	8.2
			Control soil			
B	18.9	9.7	12.6	19.4	5.7	33.7
A	14.7	14.6	6.0	14.7	9.5	40.5
YS	10.7	16.2	6.0	18.8	11.4	36.9

in control soil the percentage of Gram-negative organisms decreases while the percentage of Gram-positive cocci and sporulating bacteria rises.

Bacteria isolated from the soil before the experiment are characterized by a markedly higher growth intensity (Tab. 6). Similarly, on the roots, in the rhizosphere and in the control soil changes in the incidence of bacteria with different growth intensity can be encountered during the first 21 days of wheat growth. In the first two lots, i.e. on the 7th and 14th days after sowing, the highest percentage of growth-active types could be observed among the soil isolates. In further lots, however, i.e. on the 21st day of growth, the highest percentage of growth-active types was found among bacteria isolated from the root surface. Bacteria isolated from rhizosphere soil displayed the lowest percentage of growth-active types of bacteria in all lots removed.

In the same way as with nutritional requirements, during the determination of the growth intensity of bacteria of the individual morphological groups there existed differences in growth intensity not only between individual morphological groups but also within these groups according to the source of bacteria (Table 7). In the first two media, i.e. in the medium with mineral nitrogen and amino acids, the seed bacteria display the lowest growth activity in all morphological groups. The highest percentage of growth-active bacteria was observed among bacteria isolated from the soil and from the root surface. Among Gram-negative coccoid rods, Gram-variable rods, Gram-

Table 6. Intensity of growth of bacteria isolated from seeds, root surface, rhizosphere and control soil during the first stages of growth of wheat. The values are expressed as per cent bacteria exhibiting growth with transmission less than 90%.

Days after sowing	Medium B	Medium A	Medium YS
	Seeds		
	26.4	41.5	83.6
	Soil before planting		
	44.4	62.2	88.9
	Root surface		
7	53.5	64.1	79.4
14	39.1	48.3	84.9
21	63.2	62.1	86.2
Average	50.2	57.8	82.8
	Rhizosphere soil		
7	36.8	51.6	81.1
14	33.8	40.7	79.3
21	46.8	60.6	86.3
Average	37.9	48.8	80.8
	Controil soil		
7	60.1	67.5	92.0
14	60.5	71.7	95.0
21	34.1	45.9	79.3
Average	53.0	62.9	89.5

positive non-sporulating and sporulating rods, those isolated from the soil were the most growth-active. Among Gram-negative rods and Gram-positive cocci the highest percentage of growth-active types was among root isolates.

Differences between the bacterial flora of seeds and of soil (before experiment) as well as between the bacterial flora of root surface, the rhizosphere and control soil were also observed with respect to the occurrence of types with different physiological properties (Table 8). With increasing distance from the roots the occurrence of bacteria acidifying a glucose-containing medium and hydrolyzing starch decreases while the occurrence of nitrate-reducing bacteria rises. On the other hand, in the course of the first three

Table 7. Intensity of growth of morphological groups of bacteria, isolated from seeds, root surface, rhizosphere and control soil of wheat. The values are expressed as per cent of isolates with transmission less than 90%.

Origin	Medium B	Medium A	Medium YS
	Gram-negative short coccoid rods		
Seeds	38.3	46.8	85.1
Soil before planting	40.0	60.0	90.0
Root surface	56.6	64.0	82.0
Rhizosphere soil	42.8	51.7	84.4
Control soil	66.6	66.6	89.8
	Gram-negative long rods		
Seeds	20.5	38.5	80.8
Soil before planting	25.0	50.0	75.0
Root surface	45.1	54.9	79.5
Rhizosphere soil	38.4	52.0	73.5
Control soil	38.9	51.8	70.3
	Gram-variable rods		
Seeds	15.4	46.2	84.6
Soil before planting	20.0	20.0	100.0
Root surface	58.5	51.5	79.1
Rhizosphere soil	27.5	41.3	79.1
Control soil	68.1	72.9	89.9
	Gram-positive cocci		
Seeds	37.5	50.0	100.0
Soil before planting	58.8	76.6	94.1
Root surface	52.4	60.4	90.4
Rhizosphere soil	35.9	47.9	82.5
Control soil	51.1	56.8	92.8
	Gram-positive non-sporulating rods		
Seeds	25.0	37.5	87.5
Soil before planting	33.3	66.7	66.7
Root surface	34.3	44.7	81.1
Rhizosphere soil	37.1	45.6	88.4
Control soil	36.3	49.9	85.0
	Sporulating bacteria		
Seeds	20.0	20.0	100.0
Soil before planting	100.0	100.0	100.0
Root surface	48.1	59.3	85.1
Rhizosphere soil	42.8	50.0	89.2
Control soil	54.3	70.2	97.0

Table 8. Relative incidence of bacteria with various physiological properties on roots, in rhizosphere and control soil during the first stages of growth of wheat.

Days after sowing	Incidence of bacteria in %				
	Acidifying		Hydrolyzing starch	Nitrate-reducing	Liquefying gelatine
	glucose	sucrose			
			Seeds		
	85.4	37.5	39.8	16.6	—
			Soil before planting		
	48.0	32.0	56.0	32.0	66.0
			Root surface		
7	83.0	37.7	58.6	20.7	60.4
14	72.3	34.1	53.2	36.2	40.4
21	75.6	27.8	48.9	51.1	60.0
Average	76.8	32.1	52.6	38.9	55.3
			Rhizosphere soil		
7	66.7	38.9	51.8	57.4	64.8
14	61.1	25.9	50.0	38.9	51.8
21	58.8	27.8	40.2	34.0	52.6
Average	61.5	30.2	45.8	41.5	55.6
			Control soil		
7	50.0	22.9	39.6	45.8	56.3
14	82.7	76.9	57.7	65.4	75.0
21	42.6	13.0	18.5	25.9	43.3
Average	58.4	37.7	38.3	45.4	59.1

Table 9. Generic composition of bacteria of the root surface, the rhizosphere and control soil during the first stages of growth of wheat.

Number of isolates / Genera	Root surface	Rhizosphere soil	Control soil
	185	189	182
Achromobacter	1.1	4.8	3.3
Arthrobacter	9.7	14.3	6.6
Bacillus	6.5	10.1	36.3
Corynebacterium	6.0	5.3	3.8
Brevibacterium	1.6	3.2	4.9
Flavobacterium	16.2	11.1	5.0
Kurthia	3.2	0.5	1.7
Micrococcus	10.8	7.9	12.6
Mycococcus	4.3	8.5	12.6
Mycobacterium	8.1	7.9	3.9
Mycoplana	4.3	11.1	2.2
Pseudomonas	22.7	14.3	7.1
Vibrio	4.3	0.5	0.0

weeks of wheat growth the incidence of bacteria acidifying glucose and sucrose and hydrolyzing starch decreases on the roots while the nitrate-reducing bacteria content increases. This trend is also marked in rhizosphere soil, an exception being formed by nitrate-reducing bacteria whose occurrence in this zone decreases.

Table 9 shows the generic composition of bacterial flora isolated from the root surface, from the rhizosphere and control soil. Bacteria isolated in all three removals are comprised in the Table. It was shown that on wheat roots bacteria of the genera *Pseudomonas* and *Flavobacterium*, further *Micrococcus* and *Mycobacterium* are most abundant during the initial stages of growth. In the rhizosphere soil, the highest incidence of *Pseudomonas* and

Arthrobacter and further of *Flavobacterium* and *Mycoplana* species could be noted. In the control soil, however, bacilli and members of the genera *Micrococcus* and *Mycococcus* are most abundant. Bacteria of the genera *Pseudomonas*, *Flavobacterium* and *Mycobacterium* are considered to be typical root forms as they are not only most abundant on the roots but their occurrence decreases with increasing distance from the root. On the other hand, with increasing distance, the occurrence of *Bacillus*, *Mycococcus* and *Brevibacterium* rises. The rhizosphere soil is most characterized by bacteria of the genera *Arthrobacter*, *Achromobacter* and *Mycoplana*. But even within the individual genera different species occur in different zones. Thus, of sporulating microorganisms, the following species occurred on the roots: *Bacillus circulans*, *Bacillus brevis*, *Bacillus laterosporus*, *Bacillus pumilus*, *Bacillus sphaericus*; the following could not be found although they occurred in the control soil: *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus cereus*.

DISCUSSION

It can be concluded on the basis of several previous papers and of the results described here that the rhizosphere effect can be observed as early as during the first days of growth of plant roots. Timonin (1940) sowed surface-sterile seeds into soil and on the third day after germination he observed markedly higher numbers of bacteria in the rhizosphere than in the soil. Rovira (1956) grew non-sterile seeds of oats and tomato in sterile sand or on glass beads and he showed that the seed microflora can form a quantitatively characteristic rhizosphere population. On the basis of his paper the conclusion was drawn that the rhizosphere microflora has its origin in the seed microflora. Other authors also ascribe an important role to the seed microflora where the rhizosphere population was concerned (Rempe, 1951; Wallace & Lochhead, 1951; Pantos, 1957). On the other hand, however, some authors ascribe a more significant role in the origin of the rhizosphere population to the soil microflora (Isakova, 1939; Shilova, 1955). It was shown recently by Rouatt (1959) that in wheat it is possible to determine a marked rhizosphere effect both as concerns the number of bacteria and the incidence of groups of bacteria with different physiological properties three days after planting. He also points out that most rhizosphere organisms can originate in the soil.

When growing wheat in a nutrient solution, Macura (1958) found that seed bacteria can move over to the roots and colonize them. If, however, soil bacteria were added to the nutrient solution, these bacteria being physiologically more active than those from seeds, the soil types predominated on the roots. A similar finding was made in the work described here. In wheat seedlings, a marked rhizosphere effect was observed on the 7th, 14th and 21st day after sowing, both as concerns the number and composition of the bacterial population of roots and of rhizosphere soil. In all cases, a predominance of Gram-negative bacteria was observed on the roots. The higher incidence of bacteria requiring amino acids on roots than in the rhizosphere or control soil confirms the observation that the rhizosphere effect is noticeable immediately after the germination of seeds. A comparison of the properties of the bacterial flora of seeds and control soil with the properties of the bacterial flora of the root surface of seedlings showed that even if during the first days of growth the roots are found to contain bacteria typical for seeds, with further growth of the plant the percentage of characteristic types of soil bacteria in the composition of root bacterial flora rises. This was shown in changes of occurrence of various morphological and nutrition groups of bacteria. A comparison of growth of bacteria isolated from roots, from the rhizosphere and from control soil revealed that the lowest

incidence of rapidly growing strains occurs in the rhizosphere soil which appears to be connected with the finding that among the bacterial flora of this zone the complex-nutrition types of bacteria are most abundant. In control soil, bacteria with simple nutritional requirements predominate and during the first two removals the highest incidence of rapidly growing bacterial types was found here. On the roots, there is approximately an equal representation of all the three nutrition groups, the root bacteria exhibit more active growth than the rhizosphere bacteria and on the 21st day after sowing the roots were found to contain the highest number of rapidly growing strains. Similarly also the bacteria of these three zones differ with respect to occurrence of types with different physiological properties.

An analysis of generic composition of bacterial flora of the root surface and of the rhizosphere and control soil during the first 21 days of growth of wheat has also revealed qualitative differences. Bacteria of the genus *Pseudomonas*, *Flavobacterium*, *Mycobacterium* and to a lesser extent also *Vibrio* and *Corynebacterium* are characteristic for the root zone. The control soil which is not under the influence of growing roots is characterized by the occurrence of the genera *Bacillus*, *Mycococcus* and *Brevibacterium*. The rhizosphere zone was characterized in the experiments described here by the genera *Arthrobacter*, *Achromobacter* and *Mycoplana*. Representatives of the genus *Micrococcus* occurred in a rather considerable amount in all the zones.

It is known that the bacteria of the genus *Pseudomonas* are characteristic representatives of rhizosphere microflora (Clark, 1940; Starc, 1943; Krasiľnikov, 1944; Fedorov & Nepomiluev, 1954; Pantos, 1957). These bacteria are often producers of growth substances and can favourably influence the initial growth of plants (Krasiľnikov, 1944; Pantos, 1957). The occurrence of bacilli in the rhizosphere is usually considerably lower than in the control soil (Krasiľnikov, 1934; Krasiľnikov, Kriss & Litvinov, 1936; Lochhead, 1940). At the same time, there exist differences in the occurrence of the individual species of this genus in the rhizosphere and in soil. This was also observed in our experiments and it is mentioned by Clark (1940) and Berezova (1948). Interesting observations on the distribution and significance of sporulating organisms in the wheat rhizosphere were published by Vágner (1956). While in cocci and non-sporulating Gram-positive rods the rhizosphere effect is not very marked some other bacteria can be considered as characteristic for the root population. To these belong *Mycobacterium* (Khudyakov & Voznyakovskaya, 1956) *Achromobacter* (Voznyakovskaya, 1948), *Arthrobacter* (Lochhead, 1948; Pantos, 1957; Fedorov & Pantos, 1958), *Flavobacterium* (Pantos, 1957). Even if the function of these microorganisms is not yet known some papers refer to their possible significance. Thus *Flavobacterium solare* affects the growth of wheat favourably (Pantos, 1957). The finding of Sperber (1958) that *Arthrobacter*, *Brevibacterium*, *Flavobacterium*, *Achromobacter* and *Mycobacterium* isolated from the rhizosphere are able to solubilize apatite, is of interest. According to Sorokina (1958) *Mycobacterium phlei* and *Pseudomonas caudata* stimulate the germination of kok-saghyz.

The results presented here justify the conclusion that soil bacteria which are more active physiologically and from the point of view of growth than seed bacteria can considerably contribute to the rhizosphere population and can colonize the plant roots during the very first stages of plant growth.

SUMMARY

The composition and some properties of bacterial flora isolated from root surface, from the rhizosphere and control soil during the initial stages of wheat growth were studied. Isolated bacteria

were divided into groups according to their morphological properties, their nutritional requirements and growth intensity and according to some biochemical properties.

It was found that the bacterial flora of the root surface, of the rhizosphere and control soil during the initial stages of wheat development differ in the occurrence of morphological types, nutritional requirements and growth activity. The rhizosphere effect was also quite marked in the percentage of bacterial genera in the above three zones.

It was shown by comparing the properties of bacterial flora of seeds and of soil in which the wheat was grown that the seed bacteria, in common with the soil bacteria, can move over to the roots but that the physiologically more active soil bacteria become predominant and markedly affect the composition of the rhizosphere population.

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МИКРОФЛОРА РИЗОСФЕРЫ ПШЕНИЦЫ

I. Состав и свойства бактериальной флоры в первых фазах роста пшеницы

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Изучались состав и некоторые свойства бактериальной флоры, выделенной с поверхности корней, из ризосферы и из свободной почвы в начальных фазах роста пшеницы. Выделенные бактерии были разделены на группы по своим морфологическим свойствам, по требованиям к питанию, по интенсивности роста и по некоторым биохимическим свойствам.

Было установлено, что существуют различия между представителями бактериальной флоры с поверхности корней, из ризосферы и из свободной почвы в начальных фазах развития пшеницы, что касается морфологических типов, требований к питанию и активности роста. И что касается родов бактерий, представленных в этих трех зонах, заметно проявился эффект ризосферы.

Сравнение свойств бактериальной флоры семян и почвы, в которой пшеница выращивалась, показало, что бактерии семян так же, как и бактерии почвы, могут переходит на корни, но физиологически более активные почвенные бактерии получают преимущество и оказывают главное влияние на состав популяции ризосферы.