

BACTERIAL DIVERSITY IN SOIL IN RESPONSE TO DIFFERENT PLANTS, PHOSPHATE FERTILIZERS AND LIMING

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ABSTRACT

The diversity of bacterial isolates from soil in response to different plants (control, *Brachiaria ruziziensis* and *Cajanus cajan*), fertilization (control, simple superphosphate and rock phosphate) and liming (with and without lime) was evaluated. Phenotypic and physiological characteristics of the isolates were recorded and organized in a file to identify the bacteria. Among the isolates, 95% were Gram-positive and 5% Gram-negative rods. Soil cultivated with *B. ruziziensis* favored the nonsporing Gram-positive and Gram-negative rods compared to soils with *C. cajan* or uncultivated. Number of spore-forming Gram-positive rods were higher in plots with superphosphate than in unfertilized soil or soil fertilized with rock phosphate. In unfertilized plots, larger number of Gram-positive cocci and Gram-negative rods was obtained than in fertilized plots. Unlimed plots favored spore-forming Gram-positive rods, Gram-positive cocci and Gram-negative rods, while with liming a larger proportion of nonsporing Gram-positive rods was found. From 7 to 86% of the total isolates utilized different carbohydrates. The recording data used in this experiment was effective in the isolates identification, and might be useful for diagnosis of soil bacteria. *Bacillus*, *Cellulomonas*, *Rhodococcus*, *Enterobacter*, *Flavobacterium*, *Micrococcus* and *Arthrobacter* were the genera more commonly found. Bacterial diversity was enhanced in limed, unfertilized and plant cultivated plots.

Key words: bacteria, phosphate, *Brachiaria ruziziensis*, *Cajanus cajan*, liming

INTRODUCTION

Knowledge of factors that influence the composition of soil bacterial populations is important for the development of more rational methods to diagnose problems, improve crop productivity and develop bioremediation strategies (37). Among these factors, phosphorus is one of the most critical elements for plant nutrition, because it is not only a necessary nutrient itself, but it can also influence the absorption of other nutrients. As phosphorus is poorly available in many soils (27), plants and soil microorganisms compete for the limited quantities of this mineral through the processes of precipitation-solubilization and adsorption-desorption (35),

and therefore, fertilizers must be applied to achieve acceptable crop production.

The effects of fertilizers on soil bacteria have been described in literature. Kanazawa *et al.* (17) observed a variation in the total number of bacteria and fungi isolated from soils treated with chemical or organic fertilizers. The proportion of Gram-negative bacteria in the soil was found to be 7%, and 20% in the rhizosphere (15). Hasebe *et al.* (13) observed a predominance of cocci over the rods. The addition of superphosphate decreased significantly the bacteria and fungi counts in soil cultivated with corn (28).

Lawley *et al.* (22) found that the total number of bacteria did not vary in the rhizosphere of several grasses, but the bacterial

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composition was affected by the crop species, which influenced the number of chromogenic species, endospore-forming bacteria and *Pseudomonas* spp. Similar results were obtained by Miller *et al.* (26), who showed a variation in the number of coryneform bacteria and *Pseudomonas* spp. Bacteria isolated from the soil and roots of fruit trees were identified as endospore-forming Gram-positive rods and Gram-negative rods belonging to the genera *Agrobacterium*, *Pseudomonas*, and *Xanthomonas* (40).

The composition of soils bacteria of tropical regions have been studied little and deserve more attention. Not only do they differ from soils of temperate regions, but the nature of their microbial populations may be influenced by environmental factors (12). The study of the biodiversity of the living microbial community from soils cultivated with different tropical crop species and fertilized with different phosphate sources may contribute to a better understanding of the dynamic of soil microbial communities.

MATERIALS AND METHODS

Soil Treatment

The study was conducted in a dark-red latosol as previously described (4), having been applied 400kg P₂O₅ ha⁻¹ and, at the 30^o day, 60kg N ha⁻¹. The plants cultivated were the grass *Brachiaria ruziziensis* and the legume *Cajanus cajan*; the fertilizers studied were superphosphate and rock phosphate, both with or without liming. All treatments included plots without plants or fertilizers. Fifty well-isolated colonies were randomly chosen from each plot and streaked on potato dextrose yeast extract agar slants. To ensure culture purity, each isolate was re-streaked on nutrient agar plates then were transferred to nutrient agar slants.

Preliminary Tests

Colony morphology was determined after 2 to 4 days growth on nutrient agar plates incubated at 35-37°C. Each isolate was submitted to Gram staining (12) and was examined for cellular morphology and arrangement. The isolates were also examined for spore formation according to the Wirtz-Conklin method (2). In addition, the strains were streaked on nutrient agar with 50 µg/ml crystal violet (15) and submitted to the fast method using 3% KOH, to distinguish Gram-negative and Gram-positive bacteria (8). To determine motility, strains were grown in tryptic soy broth and after 18 h, or as soon as growth was evident, a loopful of the culture was examined at 400 X magnification under dark-field microscopy (21).

Biochemical tests

Most of the biochemical tests were performed according to MacFaddin (25). Each isolated colony was streaked on nutrient agar slants and incubated at 35 -37°C for up to 48 h to obtain abundant growth. The following tests were carried out for all

900 strains: oxidase and catalase production, methyl red; Voges-Proskauer; nitrate reduction; fermentation and oxidation, citrate utilization, lysine and ornithine decarboxylase, arginine dehydrolase, acid production in phenol red broth base containing 1% carbohydrate source (starch, L-arabinose, D-fructose, D-galactose, glycerol, D-glucose, lactose, maltose, mannitol, mannose, L-rhamnose, sucrose and D-sorbitol), gelatinase production (30), urea hydrolysis and indole production (29).

Additional tests

Aerobic, nonsporing, nonmotile, catalase-positive Gram-positive rods that exhibited filamentous morphology or that were fragmented into short rods or cocci, were classified in the genus *Rhodococcus* (5) and were submitted to the cAMP test along with *Listeria ivanovii* (7). Strains showing a positive cAMP test were classified as *Rhodococcus equi*. Gram-negative rods were streaked on McConkey agar and incubated according Bier (6), which provided a suitable medium for the presumptive identification of aerobic and anaerobic/facultative Gram-negative rods.

Recording the data

On the basis of the phenotypic characteristics, the isolates were classified into 6 groups (data not shown). The resulting data of 35 phenotypic (colony and cellular morphology not showed) and physiological characteristics of the species were verified using Bergey's Manual of Determinative Bacteriology (16), recorded, entered into a microcomputer, and also organized in a file using a commercially available database management program (Access; Microsoft, Calif.). The data for each isolate were checked against this file and the possible identities of strains that keyed out (certainty of identification, >90%) were noted.

RESULTS AND DISCUSSION

A preferential stimulation of endospore-forming Gram-positive rods was found regarding other bacteria examined (Table 1). Larger numbers of nonsporing, Gram-positive rods and Gram-positive cocci were found in plots cultivated with pigeon pea, while Gram-positive or Gram-negative rods were observed in uncultivated plots or plots cultivated with brachiaria grass. A larger proportion of nonsporing Gram-positive rods was found in soil fertilized with superphosphate compared to other treatments. Similarly, greater numbers of Gram-positive cocci and Gram-negative rods were observed in unfertilized plots. Unlimed plots favored the majority of bacteria, except the endospore-forming Gram-positive rods. Of the total isolates, 5.3% were Gram-negative and 94.7% Gram-positive, and among the latter, 76.3% were endospore-forming rods and 16.0% nonsporing rods. Gram-positive cocci were scarcely observed

Table 1. Variation in proportion (%) of Gram-positive and Gram-negative bacteria isolated from soil submitted to different treatments.

Soil treatments	Gram-positive			Gram-negative rods
	Spore-forming rod	Nonsporing rods	Cocci	
Without liming, uncultivated				
Unfertilized	96	2	0	2
Superphosphate	92	2	0	6
Rock phosphate	100	0	0	0
With liming, uncultivated				
Unfertilized	100	0	0	0
Superphosphate	86	14	0	0
Rock phosphate	90	4	4	2
Without liming, Brachiaria				
Unfertilized	66	10	0	24
Superphosphate	66	20	0	14
Rock phosphate	70	24	6	0
With liming, Brachiaria				
Unfertilized	62	24	6	8
Superphosphate	70	30	0	0
Rock phosphate	78	18	0	4
Without liming, Pigeon pea				
Unfertilized	54	22	16	8
Superphosphate	76	18	0	6
Rock phosphate	88	8	0	4
With liming, Pigeon pea				
Unfertilized	62	26	12	0
Superphosphate	62	28	0	10
Rock phosphate	56	36	0	8

(2.4%), exhibiting along with Gram-negative rods a low occurrence in soils when compared to the number of Gram-positive rods. The results obtained were comparable to those from other reports. Cattelan *et al.* (10) verified the predominance of spore-forming and non-spore-forming Gram-positive bacteria in soil cultivated with soy beans. Sagardoy and Salerno (33) observed 26% Gram-negative bacteria and 74% Gram-positive bacteria, of which 21% were endospore-forming rods, 48% nonsporing rods and only 5% cocci. Conversely, Rouatt and Katznelson (31) found a larger number of Gram-negative than Gram-positive bacteria in soil cultivated with wheat; however, the number of Gram-positive cocci was proportionally smaller in agreement with our results.

Carbohydrate fermentation by the isolates ranged from 7-20% for sorbitol, rhamnose and lactose, 40-56% for galactose,

mannitol, mannose, arabinose, starch, glycerol and sucrose, and 60.8-86.1% for maltose, glucose and fructose (Table 2). Similarly, Tate and Mills (36) demonstrated that lactose and sorbitol were utilized less than arabinose and starch. Contrasting results were obtained by Torvisk *et al.* (38), who found bacterial isolates with a lesser ability to ferment sucrose, maltose and starch. Arabinose was the most utilized pentose (4-76%), the largest percentage corresponding to the treatment pigeon pea-superphosphate-without liming. The hexoses most utilized were D-glucose (66-100%) and D-fructose (56-100%). Maltose (36-90%) was the disaccharide most utilized when compared to sucrose (36-78%), manitol (14-68%) and lactose (0-46%). The bacteria isolated from soils cultivated with brachiaria grass or pigeon pea utilized lactose better than did those from noncultivated soils. Among the sugar alcohols, glycerol (32-94%) was better utilized as a carbon source than manitol (14-68%) and sorbitol (0-22%). Starch was used in 6-92% of the isolates and the highest numbers were observed in uncultivated soil.

The enzymatic reactions of the isolates are shown in Table 3. Apparently, no factor studied influenced the diversity of the isolates. Isolates obtained in each treatment showed production of catalase (68-100%), gelatinase (62-100%), and oxidase, nitrate reductase and urease (4-86%). A low proportion of isolates showed indole production and positive reaction for the methyl red and Voges-Proskauer tests. The utilization of citrate as the sole carbon source was observed in 18-74% of the isolates. Only 14-86% of the isolates fermented glucose while 62-100% used it under aerobic conditions. Except for the phosphate fertilized plots, in absence of plants and liming, the other treatments showed a less than 50% proportion in the number of isolates that used L-arginine. The results of D-glucose utilization contrast with those obtained from Argentine soils where only 4% used D-glucose in oxidative metabolism and 33% in fermentative metabolism (33). However, similar results were obtained by Balkwill *et al.* (1) who obtained values of 82% and 4%, respectively.

Twenty genera were identified and 53 strains that were not identified at the genus level were recorded (Table 4). The bacterial species found for each treatment are listed in Table 5 (number corresponding to the species listed in the Table 4). The genera with more number of isolates were *Bacillus*, *Rhodococcus*, *Micrococcus*, *Cellulomonas*, *Enterobacter*, *Flavobacterium*, and *Arthrobacter*. Other genera observed included *Alcaligenes*, *Lactobacillus*, *Klebsiella*, *Aureobacterium*, *Serratia*, *Nocardia*, *Listeria*, *Brevibacterium*, *Jonesia*, *Agromyces*, *Pimelobacter*, *Erwinia*, and *Acinetobacter*. Similar observations have been reported for the composition of bacterial populations in the soil of different crops. Kloepper *et al.* (20) isolated bacteria of the genera *Bacillus*, *Flavobacterium* and *Pseudomonas* from peanut soil. Wheat soil was found to contain the genera *Achromobacter*, *Arthrobacter*, *Bacillus*,

Table 2. Profiles of carbohydrates utilization (%) by the bacteria isolated from soil submitted to different treatments.

Soil treatments	Carbohydrates												
	St	Ara	Fru	Gal	Gl	G	Lac	Mal	Ma	Man	Ra	Suc	So
Without liming, uncultivated													
Unfertilized	52	10	98	26	92	66	0	54	42	62	4	56	8
Superphosphate	72	28	98	28	94	68	10	48	36	38	32	54	8
Rock phosphate	84	36	94	40	24	10	2	86	32	62	2	50	6
With liming, uncultivated													
Unfertilized	92	24	96	26	40	10	0	90	14	48	2	42	0
Superphosphate	54	4	88	4	32	92	4	58	28	2	2	36	0
Rock phosphate	44	62	10	52	52	98	12	56	50	18	18	78	4
Without liming, Brachiaria													
Unfertilized	68	62	90	64	58	96	38	84	68	62	16	70	22
Superphosphate	36	58	98	26	56	92	24	40	62	26	10	78	12
Rock phosphate	42	38	88	44	48	90	20	82	40	30	6	76	8
With liming, Brachiaria													
Unfertilized	50	64	86	64	50	92	40	68	48	66	10	60	6
Superphosphate	36	72	92	36	40	74	26	46	46	46	0	52	14
Rock phosphate	50	50	82	46	46	62	22	58	32	34	4	44	0
Without liming, Pigeon pea													
Unfertilized	54	56	88	54	46	92	46	74	44	56	18	54	8
Superphosphate	54	76	90	78	54	94	30	62	46	72	16	50	12
Rock phosphate	16	36	60	28	34	74	16	40	44	60	6	48	0
With liming, Pigeon pea													
Unfertilized	6	42	64	34	34	68	30	62	34	40	16	64	8
Superphosphate	24	46	56	66	38	66	18	36	40	42	12	48	6
Rock phosphate	40	56	80	42	44	80	28	70	40	44	22	52	14

St- starch, Ara- L-arabinose, Fru- D-fructose, Gal- D-galactose, Gl- glycerol, G- D-glucose, Lac- lactose, Mal- maltose, Ma- mannitol, Man- mannose, Ra- rhamnose, Suc- sucrose, So- D-sorbitol.

Cellulomonas, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Mycococcus*, *Mycoplana*, *Nocardia* and *Pseudomonas*, with *Arthrobacter* and *Pseudomonas* being most predominant (33). Soybean soil presented greater proportions of *Bacillus*, *Pseudomonas* and *Arthrobacter* genera, with *Bacillus* being prevalent in the non-rhizosphere soil and *Pseudomonas* in the rhizosphere (10). Accordingly these results, *B. megaterium* was the predominant species.

Many studies have found treatment effects on microbial diversity or on the size of physiological groups of bacteria (9,11,18,39). However, these studies used different methods and systems, then it was impossible to directly compare to our studies. The bacterial diversity were analyzed by the fluctuations of species in the treatments. The number of species for the treatments varied from 9 to 22, demonstrating a strong influence on bacterial diversity. The largest number of species was found in limed plots (9 to 20). While the number of species ranged

from 13 to 22 and 12 to 18 in plots cultivated with brachiaria grass and pigeon pea, respectively, this number in uncultivated plots varied from 9 to 14. Thus, 20 species were not found in the uncultivated soil. The differences in the bacterial composition may be due simply to the nature of the plants used (19). These results suggest that cultivation of soil increased the number of bacterial species probably due to the root excretions (31,32) that diffuse in the soil. The diversity of bacterial species in cultivated soil may be explained from a knowledge of the metabolic and growth characteristics (Tables 2-3) of the isolates (34). However, it was not possible to access how the physiological traits of the bacteria populations were affected by the cultivated soil.

In unfertilized soil were found 9 to 22 species, which corresponded to an increase from 15 to 21 % over fertilized

Table 3. Properties of the bacteria isolated from soil submitted to different treatments^(*).

Soil treatments	Physiological reactions													
	Ox	Ca	Ge	Ni	Ur	I	MR	VP	Ci	O	F	LD	OD	AD
Without liming, uncultivated														
Unfertilized	66	98	100	92	86	2	20	14	60	62	14	8	2	54
Superphosphate	4	92	100	94	80	0	2	20	64	72	24	12	6	64
Rock phosphate	28	96	100	62	68	0	6	10	34	100	32	0	0	68
With liming, uncultivated														
Unfertilized	10	100	98	94	32	0	40	30	26	100	40	8	4	42
Superphosphate	86	100	96	86	8	0	4	6	58	92	44	0	0	44
Rock phosphate	36	98	98	60	10	0	54	28	52	98	80	0	2	30
Without liming, Brachiaria														
Unfertilized	56	72	72	98	60	0	56	28	74	94	86	18	8	32
Superphosphate	46	94	80	98	20	10	24	40	44	92	70	18	2	2
Rock phosphate	16	82	62	76	26	0	32	12	68	90	84	0	0	14
With liming, Brachiaria														
Unfertilized	34	90	76	46	34	0	56	24	74	92	80	6	2	14
Superphosphate	46	94	68	64	20	0	32	26	60	74	68	0	0	4
Rock phosphate	86	92	64	52	4	2	30	18	36	64	54	0	2	18
Without liming, Pigeon pea														
Unfertilized	46	96	70	46	10	0	24	18	20	92	84	4	6	48
Superphosphate	32	80	70	56	14	0	24	12	28	94	94	0	2	22
Rock phosphate	68	100	84	64	4	0	36	20	42	74	68	6	6	28
With liming, Pigeon pea														
Unfertilized	12	80	92	52	26	0	24	10	18	68	58	12	0	28
Superphosphate	40	82	84	86	26	0	26	26	56	66	64	4	2	14
Rock phosphate	20	68	76	80	40	0	58	38	52	82	80	0	2	30

Ox- oxidase, Ca- catalase, Ge- gelatinase, Ni- nitrate reductase, Ur- urease, I- indol, MR- metil red, VP- Voges- Proskauer, Ci- Simon's citrate, O- oxidation, F- fermentation, LD- L-lysine Decarboxylase, OD- L-ornithine Decarboxylase, AD- L-arginine Dehydrolase. ^(*) % of the total.

Table 4. List of recognized bacteria that may be among the isolates according to physiological analysis.

	No. of isolates		No. of isolates		No. of isolates
1. <i>Acinetobacter</i> sp,	1	18. <i>Bacillus megaterium</i> ,	180	35. <i>Klebsiella pneumoniae</i> ,	6
2. <i>Agromyces ramosus</i> ,	2	19. <i>Bacillus mycoides</i> ,	24	36. <i>Lactobacillus</i> sp,	6
3. <i>Alcaligenes</i> sp,	9	20. <i>Bacillus polymixa</i> ,	24	37. <i>Listeria murrayi</i> ,	3
4. <i>Arthrobacter</i> sp,	11	21. <i>B. psychrosaccharolyticus</i>	44	38. <i>Micrococcus kristinae</i> ,	2
5. <i>Aureobacterium</i> sp,	5	22. <i>Bacillus pumilus</i> ,	12	39. <i>Micrococcus luteus</i> ,	3
6. <i>Bacillus badius</i> ,	8	23. <i>Bacillus subtilis</i> ,	5	40. <i>Micrococcus sedentarius</i> ,	7
7. <i>Bacillus brevis</i> ,	62	24. <i>Bacillus thuringiensis</i> ,	52	41. <i>Micrococcus varians</i> ,	10
8. <i>Bacillus carotarum</i> ,	2	25. <i>Brevibacterium iodinum</i> ,	2	42. <i>Nocardia</i> sp,	3
9. <i>Bacillus cereus</i> ,	76	26. <i>Brevibacterium linens</i>	1	43. <i>Pimelobacter jensenii</i>	1
10. <i>Bacillus circulans</i>	61	27. <i>Cellulomonas</i> sp,	23	44. <i>Rhodococcus equi</i> ,	13
11. <i>Bacillus coagulans</i>	5	28. <i>Enterobacter agglomerans</i> ,	6	45. <i>Rhodococcus</i> sp	19
12. <i>Bacillus firmus</i> ,	31	29. <i>Enterobacter cloacae</i> ,	8	46. <i>Serratia ficaria</i>	2
13. <i>Bacillus larvae</i> ,	8	30. <i>Enterobacter nimipressuralis</i> ,	1	47. <i>Serratia marcescens</i>	1
14. <i>Bacillus laterosporus</i> ,	6	31. <i>Erwinia ananas</i> ,	1	48. <i>Serratia</i> sp	1
15. <i>Bacillus lentus</i> ,	70	32. <i>Flavobacterium meningosepticum</i> ,	1	49. RNSGPR (NI) ¹	41
16. <i>Bacillus liqueniformis</i> ,	2	33. <i>Flavobacterium odoratum</i> ,	9	50. INSGPR (NI) ²	9
17. <i>Bacillus macerans</i> ,	16	34. <i>Jonesia denitrificans</i> ,	2	51. NFGNR (NI) ³	3

1- Regular nonsporing Gram-positive rods, 2- Irregular nonsporing Gram positive rods, 3-Non fermenters Gram-negative rods.

Table 5. Bacterial species number isolated from soil submitted to different treatments.

Soil treatments			Species found per treatment*																					
P ₀	F ₀	L ₀	1	5	7	9	11	14	15	18	21	22	24											
P ₀	F _{SP}	L ₀	6	7	9	10	15	18	19	23	24	28	46	48	50									
P ₀	F _{RP}	L ₀	9	10	12	14	15	18	21	23	24													
P ₀	F ₀	L ₁	7	9	10	11	12	15	18	19	24													
P ₀	F _{SP}	L ₁	7	9	10	12	13	15	18	24	27	44	49											
P ₀	F _{RP}	L ₁	9	15	16	17	18	19	20	21	22	24	30	40	41	42								
P _B	F ₀	L ₀	3	4	9	10	13	15	17	18	19	20	21	24	27	28	29	33	35	45	47	49		
P _B	F _{SP}	L ₀	3	5	7	9	10	17	18	20	21	22	24	27	29	31	33	45	49					
P _B	F _{RP}	L ₀	9	10	18	19	20	21	22	24	27	36	41	45	49									
P _B	F ₀	L ₁	3	5	7	9	10	12	13	15	17	18	19	20	21	24	25	29	40	41	44	45	49	50
P _B	F _{SP}	L ₁	4	7	9	10	16	17	18	19	20	21	23	36	44	45	49	50	51					
P _B	F _{RP}	L ₁	3	4	6	7	9	10	15	17	18	21	24	27	31	36	49							
P _G	F ₀	L ₀	6	7	8	9	10	12	14	17	18	20	27	28	34	38	39	41	49	50				
P _G	F _{SP}	L ₀	3	7	9	10	11	12	13	17	18	21	27	46										
P _G	F _{RP}	L ₀	4	7	9	10	12	13	17	18	20	21	24	33	42	49								
P _G	F ₀	L ₁	2	4	7	8	9	10	13	18	20	21	24	26	27	40	41	43	45					
P _G	F _{SP}	L ₁	3	7	9	10	18	20	21	27	33	44	45	49	50	51								
P _G	F _{RP}	L ₁	4	9	10	11	18	20	21	27	29	36	37	44	45	49	50							

P₀ - without plant, P_B - Brachiaria, P_G - Pigeon pea, F₀ - without fertilizer, F_{SP} - superphosphate, F_{RP} - rock phosphate, L₀ - without liming, L₁ - with liming; (*) The number related in this table corresponds to that in Table 4.

plots, suggesting that phosphate fertilization had a negative effect on bacterial diversity. Accordingly these results, Lima *et al.* (23) found a higher number of bacteria in unfertilized soil

compared to soil fertilized with superphosphate. Differently, Martyniuk and Wagner (24) found that microbial populations were greater in fertilized soil than in unfertilized soil. However,

organic phosphorus decreased in the soil fertilized with superphosphate or rock phosphate when compared with the control (3). Therefore, it is possible that the limited content of organic phosphorus had decreased the number of bacterial species in the soil fertilized with phosphates.

In conclusion, the results above indicated that the recording data used in this experiment were effective in the identification of soil bacteria, and might be useful for soil diagnosis of soil bacterial isolates. These results suggest that specific bacteria were found in soil and their diversity might fluctuate depending on plant type, phosphate fertilizer and liming.

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RESUMO

Diversidade bacteriana do solo em resposta a diferentes plantas, fertilizantes fosfatados e calagem

Este estudo avaliou a diversidade de bactérias isoladas do solo em resposta a diferentes plantas (controle, *Brachiaria ruziziensis* e *Cajanus cajan*), fertilizantes (controle, superfosfato simples e fosfato de rocha) e calagem (com e sem calcário). As características fenotípicas e fisiológicas dos isolados foram registradas e organizadas em um arquivo para identificar as bactérias. Entre os isolados, 95% foram de bactérias Gram positivas e 5% bacilos Gram negativos. *B. ruziziensis* favoreceu mais os bacilos Gram positivos não esporulados e bacilos Gram negativos que o solo com *C. cajan* ou não cultivado. O número de bacilos Gram positivos esporulados foi superior nas parcelas fertilizadas com fosfato do que nas não adubadas ou fertilizadas com fosfato de rocha. Nas parcelas não fertilizadas, maior número de cocos Gram positivos e bacilos Gram negativos foi obtido do que nas parcelas fertilizadas. A ausência de calagem favoreceu os bacilos Gram positivos esporulados, cocos Gram positivos e os bacilos Gram negativos, enquanto que, com calagem foram encontradas proporções maiores de bacilos Gram positivos não esporulados. De 7 a 86% do total de isolados utilizaram diferentes carboidratos. O registro dos dados utilizado neste experimento foi efetivo na identificação dos isolados e pode ser útil para o diagnóstico das bactérias do solo. Os gêneros mais frequentes foram *Bacillus*, *Cellulomonas*, *Rhodococcus*, *Enterobacter*, *Flavobacterium*, *Micrococcus* e *Arthrobacter*. A diversidade bacteriana foi aumentada nas parcelas com calagem, não fertilizadas e cultivadas com plantas.

Palavras-chave: bactéria, fosfato, *Brachiaria ruziziensis*, *Cajanus cajan*, calagem.

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