Variation in Indigenous Rhizobium meliloti Populations and the Effect of Inoculation

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The population biology and ecology of Rhizobia are among the fields of great interest for those engaged in Rhizobium research. These types of investigations are important because of the discovery of the biological background of the competition for nodulation between bacteria and because, on the basis of the knowledge that can be acquired this way, it is possible to increase the efficiency of inoculation. The genetical variability of indigenous Rhizobium populations is well known /BROMFIELD, 1984; and BROMFIELD et al., 1986/. This variability depends, to a great extent, on the abiotic environmental factors, such as: soil type, temperature, humidity, etc. Among the biotic factors it is necessary to mention the preferences of the hosts which have a changing effect on the bacterial populations /THURMAN and BROMFIELD, 1988/.

In these experiments the variability of indigenous Rhizobium meliloti population was followed by bacteriophage typing in samples from different localities of the country and the effect of a single massive inoculation on the original population was examined.

Materials and methods

Sampling of soils with the indigenous Rhizobium meliloti populations: Three soils under alfalfa were sampled from Kompolt /K/, Putnok /P/, and Nagyhörcsök /H/. Ecologically these Hungarian localities are quite dissimilar with different soil types, and climatic conditions.

The experiments were performed in 5 replications with pots each holding 1 kg soil which had been collected without any preparation to maintain the original Rhizobium population. In the course of inoculation $2 \cdot 10^9$ Rhizobium meliloti 41 /SZENDE and ÖRDÖGH, 1960/ bacteria were spread over the soil surface and 50 sterilized Medicago sativa seeds per pot were sown and covered with soil.

After 60 days the plants were harvested, the nodules collected, the surface sterilized and the bacteria isolated, in the usual way separately, and purified, then submitted to bacteriophage typing. Bacteria were maintained in YMA medium. The inoculant and the indicator was R. meliloti 41 and was cultivated in YM broth. Bacteriophages were isolated from nodules

or from soil. For comparison the 16-3 phage was used /ÖRDÖGH and SZENDE, 1961/. This phage has a very narrow spectrum and infects only the R. meliloti 41 strain. The phages were virulent or clear plaque mutants of temperate phages. For the isolation, purification, and maintenance of phages the methodology of ADAMS /1959/ was used.

Bacteriophage typing: The phages in the experiments were printed with a sterile replicator on the bacteria in a double layer medium and tested for sensitivity against them. The sensitive bacteria are lysed after 24 h at 28°C occurring as a clear spot on the bacterial lawn /ÖRDÖGH and SZENDE, 1961; SZENDE, 1987; LESLEY, 1982/.

Media: Yeast mannitol agar /YMA/ was used in the experiments, for the inoculant bacteriophage and indicator propagation YM broth was applied.

Results and discussion

The distribution of phage types was followed in bacteria isolated from 110 nodules in case of the indigenous population and from 109 after inoculation.

In the populations 79 phage types were demonstrated. From the technical point of view, however, a wobbling was observable in the sensitivity pattern. This occurs in the case of the R. meliloti 41 strain. The original strain is the No. 21 type, but in the inoculated populations No. 46-50, the 61-66, 68, 76-79 isolates were nearly similarly sensitive /resistant to one or two tester phages in combination/. This means that the number of phage types is lower. When only the inoculant is sensitive to the 16-3 phage the difference between inoculant and indigenous bacteria is simply demonstrable.

The difference in phage types was not significant in case of indigenous populations originating from areas of different ecological conditions /Table 1/. The dominant types were the No. 4, 10, 20 at Kompolt /K/, the No. 4, 10 at Nagyhörcsök /H/ /nearly the same/. Only the No. 1 was dominant at Putnok /P/. The variation in phage type was heterogeneous. The bacteria from two nodules from Nagyhörcsök and the other from Putnok were similar or nearly similar to the R. meliloti 41 strain /Table 2/. After inoculation the population shifted to the inoculant type /Table 3/. The dominant type was the No. 21 /the inoculant type/. At Kompolt 46, 47, and 48 were similar to the inoculant type. At Putnok the dominant type was No. 48 and the population was quite homogeneous. At Nagyhörcsök there were no dominant types and the population was very heterogeneous.

It is interesting that after inoculation the ratio of the inoculant or similar types were: After inoculation: H: 0.1194; K: 0.1930; P: 0.2202; In indigenous: H: 0.0183; K: 0.0092; P: 0.0092; - calculated on the basis of 219 nodules -.

The increasing number of inoculant types was dissimilar from 29 to 100 per cent, for which probably the ecological differences /soil/ were responsible. Although the inoculation was a massive one, the increase was not absolute: 53 per cent of the 219 nodules was initiated by the inoculant type.

After inoculation hybrid types were also found in the population. This means that those types which are characteristic of the indigenous population, such as No. 4, 10 and 20 form hybrid-like types with the introduced inoculant type /No. 21/. In this case the sensitivity to the 16-3 phage is combined with those which do not lyse the inoculant. The number of such hybrid types were: K: 0.1284; H: 0.0183; P: 0.000. In case of the indigenous populations only one nodule /0.0092/ originated from H was observed as such hybrid type.

It is not exluded that these hybrid types are the result of genetic exchange between the two types /indigenous and introduced/ in the soil.

Table 1

Distribution of phage types in the indigenous and inoculated populations

Phage types	Indigenous			Inoculated			Phage types	Indigenous			Inoculated		
	K	P	H	K	P	H		K	P	H	K	P	H
1	3	3	1				41			1			
2		1					42			1			
3		1					43			1			
4	4	1	5	2		1	44			1			
5	1	1	1				45			1			1
6		1					46				1	4	
7		1					47				1	5	1
8		2				1	48				3	11	1
9		2				2	49				1	1	2
10	10	2	8	1		3	50				2	1	
11		1					51				2		
12	1	1	2				52				1		
13		1					53				2		
14		1					54				3		
15		2					55				1		
16	2	1					56				1		
17	2					1	57				2		2
18	2					1	58				1		
19	1						59				1		
20	5		3			1	60				2		1
21	1			9	2	4	61				1		
22	1		1				62				1		
23	1						63				1		
24	1	1	1				64				1		
25	1						65				1		
26	2						66				1		
27	2						67						1
28	1					1	68						1
29	1						69						1
30	2						70						1
31	1	1		1			71						2
32							72						1
33	1						73						3
34			4	1			74						1
35			2				75						1
36			1				76						1
37			1			1	77						1
38		1				1	78						1
39		1					79						1
40		1											

K: Kompolt; P: Putnok; H: Nagyhörcsök.
The differences in the distribution of the indigenous population in different ecological conditions /soils/ were not significant, calculated using Chi-squared analysis /P > 10/. After inoculation the difference in phage types occupying the nodules was highly significant /P < 0.001/.

Table 2

Frequency distribution of phage types in indigenous populations

Phage types	Kompolt	Putnok	Nagyhörcsök
1	0.0275x	0.0275x	0.0092
2	0	0.0092	0
3	0	0.0092	0
4	0.0367x	0.0092	0.0459x
5	0.0092	0.0092	0.0092
6	0	0.0092	0
7	0	0.0092	0
8	0	0.0183	0
9	0	0.0133	0
10	0.0917x	0.0183	0.0734x
11	0	0.0092xx	0
12	0.0092	0.0092	0.0183
13	0	0.0092	0
14	0	0.0092	0
15	0	0.0183	0
16	0.0183	0.0092	0
17	0.0183	0	0
18	0.0183	0	0
19	0.0092	0	0
20	0.0459x	0	0.0275
21	0.0092xx	0	0
22	0.0092	0	0.0092
23	0.0092	0	0
24	0.0092	0.0092	0.0092
25	0.0092	0	0
26	0.0183	0	0
27	0.0183	0	0
28	0.0092	0	0.0092
29	0.0092	0	0
30	0.0183	0	0
31	0.0092	0.0092	0
32	0	0	0
33	0.0092	0	0
34	0	0	0.0367x
35	0	0	0.0183
36	0	0	0.0092
37	0	0	0.0092xx
38	0	0	0.0092xx
39	0	0	0.0092
40	0	0	0.0092
41	0	0	0.0092
42	0	0	0.0092
43	0	0	0.0092xxx
44	0	0	0.0092
45	0	0	0.0092

x Nearly 3 or more per cent of the population; xx R. meliloti 41 type; xxx Recombinant type

Table 3

Frequency distribution of phage types in inoculated populations

Phage types	Kompolt	Putnok	Nagyhörcsök
4	0.0183	0	0.0092
8	0	0	0.0092
10	0	0	0.0183
11	0.0092	0	0.0275x
17	0	0	0.0092
18	0	0	0.0092
20	0	0	0.0092
21xx	0.0826x	0.0183	0.0367x
28	0	0	0.0092
31	0.0092	0	0
34	0.0092	0	0
37	0	0	0.0092
38	0	0	0.0092
45	0	0	0.0092
46xx	0	0.0367x	0
47xx	0.0092	0.0459x	0.0092
48xx	0.0275x	0.1009x	0.0092
49xx	0.0092	0.0092	0.0183
50xx	0.0183	0.0092	0
51	0.0183	0	0
52xxx	0.0092	0	0
53xxx	0.0183	0	0
54xxx	0.0275x	0	0
55xxx	0.0092	0	0
56xxx	0.0183	0	0.0183
57xxx	0.0092	0	0
58xxx	0.0092	0	0
59xxx	0.0183	0	0.0092
60xxx	0.0092	0	0
61xx	0.0092	0	0
62xx	0.0092	0	0
63xx	0.0092	0	0
64xx	0.0092	0	0
65xx	0.0092	0	0
66xx	0.0092	0	0
67	0	0	0.0092
68xx	0	0	0.0092
69	0	0	0.0092
70	0	0	0.0092
71	0	0	0.0183
72	0	0	0.0092
73	0	0	0.0275x
74	0	0	0.0092
75	0	0	0.0092
76xx	0	0	0.0092
77xx	0	0	0.0092
78xx	0	0	0.0092
79xx	0	0	0.0092

x Nearly 3 or more per cent of the population; xx R. meliloti 41 type; xxx Recombinant type

Summary

In the experiments the variability of indigenous and introduced populations of Rhizobium meliloti originating from areas of different ecological conditions was followed by bacteriophage typing. It was demonstrated that in the indigenous populations the differences in the distribution of phage types were not significant and the values of the dominant types were very low. After inoculation the composition of the populations shifted to the inoculant type. This was not absolute; it only reached this 100 per cent in one soil type. In the other two samples these were 29 and 32 per cent, respectively. After inoculation hybrid types were found. Maybe these, or some of them, are the result of genetic exchanges between the bacteria.

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