WHEAT COLONIZATION BY DETERMINATE BACTERIA

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ABSTRACT: The ability to colonize wheat by determinate strain in soil, in competition with the total soil microflora, was investigated with three mutants of strain pseudomonads which contains the nptII gene, which codes for an aminoglycosidephosphotransferase, this enzyme confers antibiotic ressistance, to both neomycin and kanamycin. Number of bacteria was obtained from bulk soil, rhizosphere and rhizoplane, using phase contrast microscopy. Mutants were detected using selective agar for pseudomonas supplied with neomycin and genomic fingerprinting of bacteria used molecular genetic method ERIC-PCR. On the rhizoplane, number of all three mutants where similar at about 10^4 per 3cm root, but the total heteretroph population varied so that the proportion of mutants appeared to vary from 6.3% for PCM40074 to 20% for PCM40313, to 84% for PCM40326. This could be due occasion but by observation of 24 colonies of heterotrophs isolated on TSA, 10 were positively identified as PCM40326 using ERIC-PCR and antibiotic-resistance phenotype on selective agar and indicates that it may be more competitive than the other two mutants.

Key words: colonization, marker genes, nitrogen fixation, wheat

INTRODUCTION

Biological nitrogen fixation is very actual object of investigation of numerous scientific teams during the last 110 years. A lot of question related to nitrogen fixation of nonlegume plants and biology nature of diazotroph, were resolved but plant of small grains are supplied with nitrogen by application of mineral nitrogen fertilizer. The efficient association with significant influence on cereal plant host was not realized and transfer of *nif* genes was not commercialized, respectively (Dixon et al., 1997). Process of creation of effective association of genotype strain characterize a lot of fundamental ignorance and each new knowledge in this investigation represents a contribution to future investigation (Webster et al., 1998).

In the process of selection of small grains usually breeders do not care about association of diazotroph. Diazotrophs have influence to morphophysiological traits, biomass, yield, and containt of nitrogen in host plant (Saric et al., 1990). The high nitrogenase enzyme activity indicated that is possible cultivar determination in which rhizo-

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sphere diazotroph found advanced conditions for itself development (Micanovic et al., 1997) and increasing of nitrogen activity, biomass and nitrogen contents by passage diazotrophs method indicated availability of cultivar to make selection and multiplication of diazotroph in rhizoplan (Micanovic, 1997). Colonization on rhizoplan is a very important phase in making association. Microbial growth in the rhizosphere is stimulated by the continual input of readily assimilable organic substrates from the root (Lynsh and Whipps, 1990).

The question is which bacterial species or strain will the most efficiently use a substrate and colonized root, because of differences in process of root colonization by different bacteria, what mean that exist some kind of selective advantage (Partiquin et al., 1983).

In this work was studied possibility of application of colonization in wheat by pseudomonas competition with heterotrophic population of soil as well establishing of competition of three mutants of Pseudomonas by marker genes application. Most studies reported that pseudomonas strong root colonizing bacteria. Many isolates from this group are known to demonstrate antagonistic activity other microorganisms of the soil and rhizosphere and, therefore, have been intensively used in trials for the biological control of soil-borne plant diseases in wheat. (Miller et al., 1990). Many Pseudomonas spp. produce antibiotics which can play a role in the competition with other rhisosphere microorganisms (Letty et al., 1995). The mechanisms by which Pseudomonas spp. exert their beneficial effect on plants can be very diverse (Dowling and O'Gara, 1994). Various strains of pseudomonas have also been identified as diazotrophs (Young, 1992). That other bacteria groups or species may be present in larger numbers than have been previously reported and may play a significant role in the rhisosphere. The study of microbial interachons in the rhizosphere is dificult because of the inherent variablity in the number and distribution of microorganisms; any method developed must therefore be able to sample a large accurate and efficient. A number of techniques have been used. Several workers have used direct microscopic techniques both qualitatively and quantitatively (Foster et al., 1983). Direct microscope techniques are suitable for determining with considerable precision the level of bacterial colonization on particular areas of the root system, but in quantitative studies the high level of concentration needed by the observer makes this techniques unsuitable for processing large numbers of samples (Davies and Whitbread, 1989).

MATERIAL AND METHODS

The Apollo variety of *Triticum aestivum* species and mutants of pseudomonads RSM 4002 (PCM 40326, PCM 40313 and PCM 40074). Mutans contains *npt*II gene, which are responsible for resistance to neomycin and kanamicin both. For integrated a gene into a genome bacteria used of transposon B_{20} (T_{n5} with inserted *lac*Z gene) (Curnow, 1998). Mutans kindly suplieed by Penny Hirsch and Phil Curnow (IACR Rothamsted, England).

Seeds were surface-sterilized in 5% sodium hypochlorite and transferred on TSA, diluted to produce to ratio 1:10. Plates were incubated at 22^{o} C during two days. Inoculation of seed was made by culture 24^{h} old containig 10^{6} bacterial cells $seed^{-1}$. Plants were grown 15 days on mixture of soil on which previously grown wheat on field

and sand on 16^h of photoperiod with 300 μmol m^{-2} s^{-1} , at 18^o C of daily temperature and night at 16^o C.

For analysis were used clean root (3 cm), root with particle of soil (3 cm) and environ soil. The total heterotrophs population counting on TSA substrate and number of mutants on TSA^{+neo} (100 mg/ml). Inoculation was made with 20 ml of inoculum diuted from 10^{-1} to 10^{-6}. The samples were incubated 2 days at 28^o C.The number of bacteria was established by Petroff-Hausser counter and phase contars microscopy.

Genetic diversity of bacteria were made by molecular biological method ERIC-PCR (De Bruinj, 1992; Versalovic et al., 1994).

RESULTS AND DISCUSSION

The number of mutants in soil varied from 5×10^3 – 5×10^4 g^{-1} of soil and total heterotrophs population in ratio 2×10^4 – 2×10^4 g^{-1} of soil. In rhizosphere variation of number of mutant was higher (7×10^3 – 6×10^4 3 cm^{-1} root) than in total heterotrophs population (9×10^4 – 1×10^5 $3cm^{-1}$ root). The lowest variation of number of mutant (9×10^3 – 2×10^4 $3cm^{-1}$ root), as a total heterotrophic population (2×10^4 – 6×10^4 $3cm^{-1}$ root) have been in rhizoplant (Tab. 1). The high presence of mutant PCM 40326 was registered on the pure root where was most advantageous condition for development of bacteria. The conductive conditions have influence to increasing number of bacteria paralell to increasing of root mass (Katupitya et al., 1995; Raicevic, 1996).

Colonization was highly dependent on the region of the root sampled and this interacted with the time of harvest (Davies and Whitbread, 1989). According to Foster (1986) the highest number of bacteria exist in the root zone with root-hairs because of the most rapid multiplication of bacteria. Around the root is formed layer of polysaccharides with optimal concentration of oxygen and increased concentration of attractants which are drawing bacteria.

Table 1. Number of mutants and total heterotrophs population (g^{-1},$3cm^{-1}$)

Mutants of pseudomonads RSM 4003	Sample/ Substrate	(TSA + neo)	TSA	% mutans of heterotrophs populations
PCM 40326	Soil	1.2×10^4	1.3×10^6	2.5
	Rhizosphere	6.7×10^3	1.2×10^5	5.6
	Rhizoplane	1.5×10^4	1.7×10^4	84
PCM 40313	Soil	5.6×10^4	8.8×10^5	6.4
	Rhizosphere	1.8×10^4	8.9×10^4	20
	Rhizoplane	8.9×10^3	1.7×10^4	50
PCM 40074	Soil	4.5×10^3	2.0×10^4	22
	Rhizosphere	6.2×10^4	1.2×10^5	1.2
	Rhizoplane	7.9×10^3	6.3×10^4	6.3

However, in mutant PCM 40074 the highest number of bacteria was found in rhizosphere zone what is in agreement with results of Dobereiner and Avuda (1966), while in mutant PCM 40313 the highest number was found neighborhood soil.

Results are indicating specificity of association cultivar-strain as well as that selection needs conduct also on this level.

Within examined zones the variation were lower so the number of mutant in rhizoplane was very similar 10^4 per 3 cm root. However, the impact of mutant in total heterotrophs population highly varied from 6.3% in PCM 40074, to 50% in PCM 40313, and 84% in mutant PCM 40326. The high existance of PCM 40326 can be by pure chance, but analysis of high number of colony of heterotrophs with TSA from one plant in experiment with PCM 40326 by using ERIC-PCR, the highest number was identified as PCM 40326 (Fig. 1).

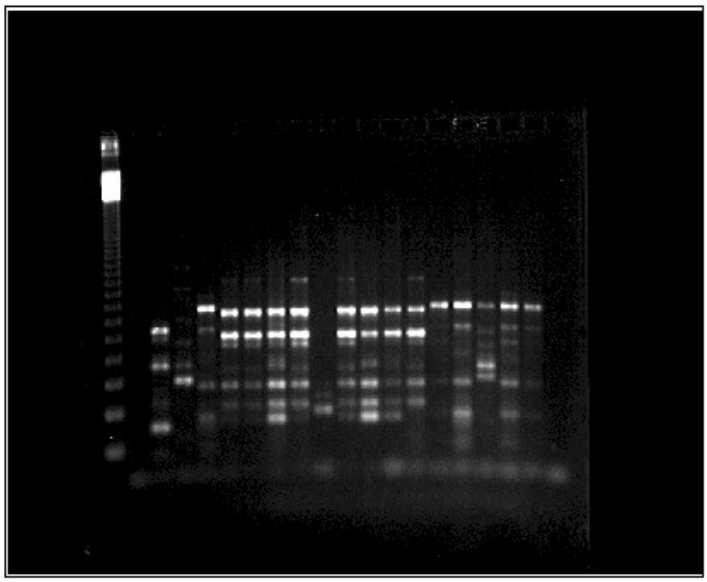

Fig. 1. Detection of mutant PCM 40326 by ERIC-PCR.
Line:1, 123 bp landder, MSM; 2, no DNA-negative control; 3-7, DNA from samples; 8, positive control - Ps. RSM 4003 DNA; 9- 19, DNA from samples.

CONCLUSION

On the root of Apolo cultivar of plant species *Triticum aestivum* was established significant number of colony of mutant PCM 40326, PCM 40313 and PCM 40074. Results indicated that there is possibility of application of his analysis for establishing of colonization availability of certain bacteria species, strain respectively, of microorganism on wheat root.

The dominance of PCM 40326 mutant in relation to other two mutants what indicated its higher competion and colonization as well as decreased competition of other two mutants in relation to total heterotrophs population of soil. The results are indicating importance of application of ERIC-PCR for identification genetic diversity of bacteria and role of marker genes in determination of certain bacterial species or strain on root plant.

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