UDC: **546.17:633.85**
original scientific paper

Acta Agriculturaae Serbica, Vol. III, 5 (1998) 29-37

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • CACAK •

Effect of Strains *Azotobacter chroococcum* and *Azospirilum lipoferum* on the Yield of Sugar Beet and Microbiological Activity of Soil

M. Govedarica
Agricultural Faculty, Novi Sad, Yugoslavia

Nada Milosevic
Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia

D. Djukic, L. Mandic
Faculty of Agronomy, Cacak, Yugoslavia

Abstract: The effect of the highly efficient strains *Azotobacter chroococcum* and *Azospirllium lipoferum* was studied on the yield of two varieties of sugar beet and on the microbiological activity of the carbonate chernozem, on which this crop was cultivated. Investigations have shown that the application of nitrogen-fixing bacteria may exert higher yield of root and crystal sugar, but at the cost of decrease in potassium, natrium and alpha-amino nitrogen in sugar beet. The use of nitrogen-fixing bacteria resulted in a higher total number of microorganisms, more numerous ammonifiers, actynomicetes, oligonitrophilic bacteria and in a stronger dehydrogenasis activity, whereas fungi decreased in number.

Key words: microorganisms, nitrogen-fixing bacteria, yield, sugar beet

Introduction

It was estimated that, in the process of biological nitrogen fixation, around 170 J 190 million tons of atmospheric nitrogen from the atmosphere can annually be fixed on the planet in all the ecosystems, using nitrogen-fixing bacteria (Babeva and Zenova, 1989). Thus, in the process of nitrogen fixation, nitrogen-fixing bacteria are able to fix 20-60 kg N per hectare of arable soil, and by some authors, even up to 150 kg (Doberainer et al. 1972; Misustin et al. 1982; Govedarica et al. 1994 and ohers). In addition, nitrogen-fixing bacteria are able to rapidly colonize and enhance on or in the plant root so that entire biological nitrogen is sent to plant. Biological nitrogen has many advantages, such as: it is more suitable than that found in mineral fertilizers, then, it travels directly (no losses) in the form suiting plants and, is the cheapest and non-contaminant to the environment.

Nitrogen-fixing bacteria can also accumulate a range of heavy metals and radioactive matter, thus, such substances are excluded from the process of cycling and do not reach plants, consequently. Due to their multiple positive effects, nitrogen-fixing bacteria are able to enhance both quantity and quality of plant production and a better microbiological activity of the soil, too.

The major objective of this study was therefore to thoroughly investigate the effect strains of *Azotobacter chroococcum* and *Azospirillium lipoferum* had on the yield of sugar beet as well as on the microbiological activity of the soil under this crop.

Material and Method

Three strains of *Azotobacter chroococcum* (84, 86, 201) and two strains of *Azospirillium lipoferum* (4,5) from the collection of the Laboratory of Microbiology of the Agricultural Faculty in Novi Sad, were used for these investigations. The effect of nitrogen-fixing bacteria was tested on two varieties of sugar beet: Dana (bred at the Institute of Field and Vegetable Crops in Novi Sad and, Alina (bred at the Institute of Sugar Beet in Aleksinac), which were cultivated in field conditions on the carbonate chernozem type of soil. Wheat served as a preceding crop. Autumnal deep ploughing up to 30 cm was performed. Before the harvest, disc harrow and seedbed conditioner were applied. The trial was set up with five replications with elemental plot 30 m2 in size. At pre-sowing, 400 kg/ha NPK (18 : 16 : 24) was ploughed in and additional foliar nutrition was performed with Murtonik. The titre of cells of *Azotobacter chroococcum* ranged from 18-35 x 106 cells per 1.0 ml of the suspension, and the titre of cells of *Azospirillium lipoferum* ranged from 10-22 x 106 cells per 1.0 ml of the suspension. The control treatment was not inoculated. Two cultivations and one digging were performed for weed control. Of the herbicides, Dual (1.5 t/ha) and Venzar (0.7 kg/ha) were single and, of the insecticides, Monokrotofost (1 l/ha) was applied, with fungicides against *Cercospora beticola* Sacc., being also performed. Impact (0.5 kg/ha) + Brestan (0.7 kg/ha) was first and Duet (1 l/ha) second applied. Alpha-amino N, Na, K and crystal sugar were determined using the method of Wieninger and Kubadinov (1992). The yield of sugar beet was expressed in tons and that of crystal sugar in kilos per hectare. Digestion was determined according to JUS E V1080 II, which has been in use since 1967.

The samples for microbiological analysis were taken in a sterile manner between the rows of beet at the depth of 10-30 cm. The number of microorganisms was determined using standard microbiological methods after Pochon and Tardieux (1962), Krasiljnikov (1970), an indirect method of dilution applying an appropriate dilution of soil suspension to appropriate nutritional bases. Dehydrogenasis activity was determined spectrophotometrically after Lenhard (1956) and modified after Thalman (1968).

Results and Discussion

It was established that bacteriyation of beet seed with nitrogen-fixing bacteria could enhance, both root yield and its sugar content, as well as the soil microbiological activity.

The strains involved *Azotobacter chroococcum* and *Azospirilium lipoferum* indicated an outstanding efficiency in both varieties of sugar beet (Tab. 1 and 2).

Tab 1. Effect of diazotrophes on yield and quality of sugar beet variety Dana

Varieties	Yield root (t/ha)	Digestion (%)	K	Na	α-amino-N	White sugar yield (kg/ha)
				m mola/100g		
Control	54,8	14,7	4,40	1,70	3,80	6600,0
Azotobacter chroococcum strain-84	75,0	16,0	4,50	1,25	2,05	7200,2
Azotobacter chroococcum strain-86	64,0	16,4	4,30	1,40	2,04	7111,0
Azotobacter chroococcum strain-201	69,0	15,5	4,30	1,30	2,20	8490,1
Azospirillum lipoferum strain 4	74,0	15,5	4,21	0,95	2,29	9400,0
Azospirillum lipoferum strain-5	54,0	15,2	4,30	0,97	2,20	8000,0

Tab. 2. Effect of diazotrophes on yield and quality of sugar beet variety Alina

Varieties	Yield root (t/ha)	Digestion (%)	K	Na	α-amino-N	White sugar yield (kg/ha)
				m mola/100g		
Control	55,3	13,8	5,50	0,95	4,00	6190,0
Azotobacter chroococcum strain-84	58,0	15,0	5,04	0,80	3,00	6800,0
Azotobacter chroococcum strain-86	64,4	15,6	4,60	0,80	2,80	8500,4
Azotobacter chroococcum strain-201	65,5	15,4	4,60	0,95	2,00	7945,3
Azospirillum lipoferum strain 4	61,0	15,9	4,45	0,90	1,84	7511,1
Azospirillum lipoferum strain-5	63,4	14,0	4,55	0,80	2,00	5499,9

Thus, the highest efficiency in the variety Dana was obtained using azotobacter, strain 201 and azospirilum, strain 4. In the variety Alina, the highest efficiency was exerted using azotobacter, strain 86 and azospirilum, strain 5. The yield and its quality were also enhanced in other plant species (Doberainer et al. 1972; Umarov, 1982; Feodorova, 1990; Govedarica et al., 1994, 1996; Jarak et al. 1998). According to these longterm studies, the use of various nitrogen-fixing bacteria has considerably increased beet and its sugar yield (Govedarica et al., 1997a, b, c; Milosevic et al., 1997a, b; 1998a, b, c). Antipcuk et al. (1997) reported that the use of azotobacter in beet production might enhance root yield and its crystal sugar content by 5900 kg/ha and 1000 kg/ha, respectively. However, such values were proven much higher in the present studies. On the other hand, the use of nitrogen-fixing bacteria also caused a decrease in potassium, natrium and alpha-amino nitrogen in the root (Tab. 1 and 2). On the average, nitrogen-fixing bacteria indicated the best efficiency in the variety Dana and somewhat poorer one in Alina. Introduction of nitrogen-fixing bacteria with beet seed brought about an increase in the total of microorganisms (Tab.3), actynomicetes (Tab.4), azotobacter (Tab.5), oligonitrophilic bacteria (Tab.5) and dehydrogenasis acivity (Tab.6) and, a decrease in the number of fungi. On the average, it was in the variety Dana that nearly all the test groups of microorganisms appeared in the highest number, whereas in Alina they appeared in a lower one.

Based upon these and many other investigations, it is evident that nitrogen-fixing bacteria are to be selected on the level of a plant genotype which will enable the most efficient strains to be bred (Umarov, 1983; Zavjaginicev, 1985; Vasjuk, 1987; Govedarica et al. 1990, 1994, 1996, 1997, 1998). Further use of nitrogen-fixing bacteria in beet production would make it much more profitable with a highly valuable product ensured.

Tab.3. Effect of Nitrogen-fixing Bacteria on the Total of Microorganisms and the Number of Ammonifiers in Soil under Sugar Beet

Varieties	Total number of microorganisms (10^6/g soil)		Ammonifiers (10^6/g soil)	
	Dana	Alina	Dana	Alina
Control	590.4	585.5	480.8	475.7
Azotobacter chroococcum strain 84	740.3	620.2	622.2	640.1
Azotobacter chroococcum strain 86	820.1	711.2	670.3	590.9
Azotobacter chroococcum strain 201	940.4	820.4	820.2	680.1
Azospirillum lipoferum strain 4	900.0	700.0	690.9	590.9
Azospirillum lipoferum strain 5	880.2	711.1	711.1	670.2

Tab.4. Effect of Nitrogen- fixing Bacteria on the Total of Fungi and Actynomicetes in Soil under Sugar Beet

Varieties	Fungi (10^4/ g soil)		Actinomycetes (10^5/ g soil)	
	Dana	Alina	Dana	Alina
Control	20.4	19.2	21.0	20.4
Azotobacter chroococcum strain 84	12.2	11.2	40.2	35.5
Azotobacter chroococcum strain 86	13.1	10.1	50.2	60.2
Azotobacter chroococcum strain 201	9.1	8.9	60.2	50.1
Azospirillum lipoferum strain 4	8.2	7.1	62.4	30.9
Azospirillum lipoferum strain 5	14.1	14.8	50.0	40.1

Tab.5. Effect of Nitrogen-fixing Bacteria on the Number of Azotobacter and Oligonitrophilic Bacteria in the Soil under Sugar Beet

Varieties	Azotobakter (10^2/ g soil)		Oligonitrofilne bakterije (10^2/ g soil)	
	Dana	Alina	Dana	Alina
Control	20.8	20.0	280.1	190.9
Azotobacter chroococcum strain 84	41.0	30.0	620.2	400.3
Azotobacter chroococcum strain 86	60.2	52.2	599.8	590.9
Azotobacter chroococcum strain 201	94.0	74.4	980.0	490.2
Azospirillum lipoferum strain 4	75.0	62.0	740.0	411.1
Azospirillum lipoferum strain 5	62.0	59.0	700.0	577.7

Tab.6. Effect of Azotobacter on Dehydrogenasis Activity of Soil under Sugar Beet

Varieties	Dehydrogenase activity (μg TPF/ 10 g soil)	
	Dana	Alina
Control	350.0	280.0
Azotobacter chroococcum strain 84	750.0	650.0
Azotobacter chroococcum strain 86	700.0	600.0
Azotobacter chroococcum strain 201	990.0	590.0
Azospirillum lipoferum strain 4	840.0	650.0
Azospirillum lipoferum strain 5	550.0	680.0

Conclusion

The use of nitrogen-fixing bacteria in beet production increased beet yield and microbiological activity of soil, too.

The efficiency of azotobacter was found to depend on the species and strain as well as on the variety of sugar beet.

Azotobacter, strain 201 and azospirilum, strain 4 reached the best efficiency in the variety Dana, whereas azotobacter, strain 86 and azospirilum, strain 5 achieved the best one in Alina.

Introduction of nitrogen fixers with beet seed led to a higher total of the miroorganisms, ammonifiers, azotobacter, oligonitrophilic bacteria and dehydrogenasis activity, but to a smaller number of fungi.

In both varieties studied, bacterization of the beet seed with nitrogen-fixing bacteria caused lower rates of potassium, natrium and alpha-amino nitrogen in the root.

On the average, the variety Dana indicated the best efficiency and the strongest microbiological activity, whereas Alina indicated somewhat poorer ones.

References

Antipčuk, F. A., Rangelova, M. V., Tancjurenko, V. O., Ševčenko, I. A. (1997): Vpliv azotobaktera na vrošaj ta jakist curkovih burjakiv. Mikrobiologičeskij Žurnal, Tom 59,No 4, 90-95.

Babeva, I. P. Zenova, A. R. (1989): Biologija počv. Izd. Moskovskogo Univerziteta. Moskva.

Dõberainer, J, Daj, J. M., Dart, P.J. (1972): Nitrogenase activity and oxygen senzitivity of the *Paspalum notatum* Đ *Azotobacter paspalii* association. J. Gen. Microbiol. 71,No 1, 103-106.

Dõberainer, J. (1978): Influence of environmental factor on the occurrence of *Spirillum lipoferum* in soil and roots Ecol. Bull. No 26, 343-351.

Govedarica, M. (1986): Azotofiksatori i njihova aktivnost kod kukuruza. Doktorska disertacija, Poljoprivredni fakultet, Novi Sad.

Govedarica, M. (1992): Efektivnost azotobaktera kod nekih hibrida kukuruza. Savremena poljoprivreda,Vol. 40, Broj 6, 87-93.

Govedarica, M., Jeličić Zora, Milutinović Vera, Jarak Mirjana, Milošević Nada, (1992): Ispitivanje efektivnosti azotofiksatora kod kukuruza. Zbornik Matice srpske za prirodne nauke, Sv. 82, 119-122.

Govedarica, M., Jeličić Zora, Jarak Mirjana, Milošević Nada, M. Pavlović (1994): Ispitivanje efektivnosti različitih vrsta azotobaktera kod kukuruza. Zbornik radova devetog savetovanja agronoma i tehnologa. 14-16.02., Smederevo, 44-47.

Govedarica, M., Jeličić Zora, Jarak Mirjana, Milošević Nada, M. Pavlović (1996): Diazotrofi i njihova aktivnost kod kukuruza. . Zbornik radova jedanaestog međunarodnog savetovanja agronoma i tehnologa, 13-16.02., Aranđelovac, 153-159.

Govedarica, M., Jeličić Zora, Jarak Mirjana, Milošević Nada, M. Pavlović (1997): Mogućnost primene biološkog azota u proizvodnji kukuruza. Zbornik radova jedanaestog međunarodnog savetovanja agronoma i tehnologa, 13-16.02. Aranđelovac, 69-77.

Govedarica, M., Jeličić Zora, Jarak Mirjana, Milošević Nada, Stojnić Nada, D. Rašković (1998): Značaj asocijativne azotofiksacije u proizvodnji kukuruza. Zbornik naučnih radova, 4, 95-101.

Govedarica, M., Milošević Nada, Jarak Mirjana, (1997a): BioloŠka azotofiksacija: Mogućnosti, primena i perspektive. Zbornik radova Naučnog instituta za ratarstvo i povrtarstvo, sv. 29, 27-35.

Govedarica, M., Milošević Nada, Jarak Mirjana, Đurić Simonida, Petrović, N., Milošev, D., Krunić Vesna, Kuzevski Janja, (1997b): Isptivanje efektivnosti azotobaktera kod šećerne repe. Letopis naučnih radova Poljoprivrednog fakulteta, godina 20, br. 2, 3-40.

Govedarica, M., Đurić Simonida, Stamenković-Jovanović Snežana, Sekulić Gordana, Antić Tamara, (1997c): Perspektive biološkog azota u biljnoj proizvodnji. Uređenje, korišćenje i očuvanje zemljišta. 9 kongres JDPZ, jun, 399-404.

Fedorova, L. S., Pozonjakova, L., Kanevskaja, S. V. (1985): Videlenie Azospiril iz kulturnih i dokorastucih zlakov saratovskoj oblasti. Mikrobiologija, T 54, Nauka, 684-685.

Krasiljnikov, N. A. (1970): Opredelitelj bakterij i aktinomicetov. Izd. ANSSSR, Moskva.

Jarak, M., R. Protić, M. Govedarica, Nada Milošević, N. Urić (1998): Uticaj agrotehničkih mera na mikrobiološku aktivnost u rizosferi pšenice. Zbornik naučnih radova, 4, 39-45.

Milošević Nada, Kuzevski Janja, Jarak Mirjana, Govedarica, M., Krstanović, S., (1997a): Azotobakter - inokulaciona tehnologija u proizvodnji šećerne repe. Zbornik naučnih radova PKB ''INI AGROEKONOMIK'', Vol 3, No 1, 109-119.

Milošević Nada, Govedarica, M., Kuzevski Janja, Jarak Mirjana, Krstanović, S., (1997b): Field testing of Azotobacter chroococcum effectiveness in sugar beet. Proceedings of the 11th International Congress on Nitrogen Fixation for the 21th Century. Paris, 20-25 july, France, 408-409.

Milošević Nada, Govedarica, M., Jarak Mirjana, Kuzevski Janja, Krstanović, S. (1998a): Uticaj sabijanja na strukturu i biogenost zemljišta pod šećernom repom. Zbornik radova PKB ''INI AGROEKONOMIK'', Vol 4, No 1, 95-103.

Milošević Nada, Govedarica, M., Kuzevski Janja, Jarak Mirjana, Krstanović, S., (1999a): Primena mikrobnih inokulanata u proizvodnji šećerne repe, mogućnosti i perspektiva. Zbornik radova PKB ''INI AGROEKONOMIK'', Vol 5, No 1, 141-151.

Milošević Nada, Govedarica, M., Jarak Mirjana, Đurić Simonida, (1999b): Effect of inoculation with Azotobacter chroococcum on Corn and Sugar beet and Biological activity in soil. Proceedings Research

(Hungary, Romania, Yugoslavia). Novi Sad, 24-25, september, Part II, 643-649.

Milošević Nada, Govedarica, M., Đukić, D., Mandić, L., (1999c): Biofertilizacija, imperativ budužnosti. Zbornik radova zimske škole agronoma. Čačak, Vol. 3, No 3, 123-129.

Mišustin, E. N., Kalinskaja, T. A., Šerbakov, M. J. (1978): Nesimbiotičeskaja azotofiksacija v pohotvih počvah. U. KN. Problemi počvovedenija, M. s. 92-96.

Pochon, J. and Tardieux, P. (1962): Techniques d' analzse en microbiologie de sol, Edit. de la Tourelle, Paris.

Thalman, A. (1968): Zur methodik der Bestimmung der Dehydrogenase activitat in boden Mittels Tripheniltetrazolium (TTC). Landw. Forsch, 21, 249-258.

Umarov, M. M., (1983): Asocijativna azotofiksacija (osobenosti, produktivnost, značenje v azotnom bilanse počve) Avtoref. dokt. dis. M.: MGU, 49 s.

Umarov, M. M. (1985): Asocijativna azotofiksacija, problemi i perspektiva. Bjulten VNII s-h. Mikrobiologiii, No 42, 9-14.

Vasjuk, F. L. (1985): Asocijativni azotofiksatori i uslovi ih efektivnovo primenenia. Bjulten VNII s.-h. mikrobiologii, No 42, 16-24.

Wieninger L., Kubadivow, N. (1992): Metode za laboratorijsku kontrolu procesa proizvodnje fabrike šećera, Tehnološki fakultet, Novi Sad.

Zavjagincev, G. D. (1985): Problemi upravlenija azotofiksatorami v rizosfere i v rizoplane. BjultenVNIIs.-h.mikrobiologii,No42,6-9.

UTICAJ SOJEVA *Azotobacter chroococcum* i *Azospirillum lipoferum* NA PRINOS ŠEĆERNE REPE I MIKROBIOLOŠKU AKTIVNOST ZEMLJIŠTA

-originalni naučni rad-

M. Govedarica
Poljoprivredni fakultet, Novi Sad

Nada Milošević
Naučni institut za ratarstvo i povrtarstvo, Novi Sad

D. Đukić, L. Mandić
Agronomski fakultet, Čačak

Rezime

Azotofiksatori su mikroorganizmi koji fiksirani atmosferski azot predaju biljci u najpovoljnijem obliku. Cilj ovih istraživanja je bio da se ispita uticaj *Azotobacter chroococcum* i *Azospirillum lipoferum* na prinos šećerne repe i mikrobiološku aktivonst zemljišta pod ovom važnom industrijskom kulturom.

Šećerna repa je uzgajana u poljskim uslovima na zemljištu tipa karbonatni černozem. Pre setve izvršena je inokulacija semena šećerne repe sa azotofiksatorima. Istraživanja su pokazala da je efektivnost azotofiksatora zavisila od primenjene vrste i soja, kao i sorte šećerne repe.

Najveća efektivnost azotobaktera kod sorte Dana dobijena je sa sojem 201, a azospiriluma sa sojem 4. Kod sorte Alina najveću efektivnost izazvao je azotobakter soj 86 i azospirilum soj 5.

Unošenje azotofiksatora sa semenom šećerne repe izazvalo je i povećanje ukupnog broja mikroorganizama, brojnosti amonifikatora, azotobaktera, oligonitrofilnih bakterija i dehidrogenazne aktivnosti, a smanjenje brojnosti gljiva.

Bakterizacija semena šećerne repe sa azotofiksatorima izazvala je kod obe sorte smanjenje količine kalijuma, natrijuma i alfa-amino azota u korenu.

U proseku, najveća efektivnost i mikrobiološka aktivnost dobijena je kod sorte Dana, a nešto slabija kod sorte Alina.