UDC: 631.461.1.635
original scientific paper

Acta Agriculturae Serbica, Vol. I, 1 (1996) 3–11

Acta Agriculturae Serbica
FACULTY OF AGRONOMY • ČAČAK •

Associative symbiosis of soil diaoztrophic bacteria and plants and its contribution to yield of vegetable crops

V. T. Emtsev,
Deparment of Microbiology, Timirjazev Agricultural Academy, Moscow, Russia

Abstract: The capacity of associative diazotrophes strains, obtained by the method of analytical selection, to permanent reproduction and growth in the rhizosphere (rhizoplane) on non-leguminous species (vegetables, first of all) was studied. It was presumed that they should conserve their initial properties, such as nitrogen fixation, phytohormones and some fungistatic agents production. The probability of positive effect of these bacteria on crops growth and production has been assessed.

Key words: bacteria, symbiosis, soil, vegetable crops

Introduction

Many scientists in various parts of the world have reported favourable responses of non-leguminous plants to inoculation with associative nitrogen fixing bacteria. The pioneer works in this field appeared a little more than fifteen years ago. The practical aspect of these works is also of great interest since plants were shown to satisfy 1/2 -1/3 of their requirements in N by associative N2-fixation. Recently there has been an increased response of vegetable crops to these bacteria.

Material and Methods

The objects of analytical selection of associative diazotrophes active strains were the rhizoplane of cucumber, tomato, pepper, lettuce and some other plants. All the associative nitrogen-fixating bacteria tested were isolated by the spermosphere model method. All the strains were maintained on Eshby's medium growth at 28 ^{0}C. (twenty eighth degree). Nitrogen fixation rates were determined with liquid cultures by acetylene reduction method.

Results and Discussion

Some 338 associations were obtained from the rhizoplane of 195 kinds and hybrids of cucumber. Facultative - anaerobic diazotrophes proved to be dominant. They are members of Enterobacteriaceae family and Klebsiella genus. Representatives of Bacillus genera were found, whereas Azotobacter, Azospirillum, Xanthobacter and Clostridium were absent. Pseudomonas bacteria were associated with diazotrophes-entero-bacteria in the microbial associations under study. Determination of nitrogenase activity by numerous associations separated from cucumber rhizoplane permitted to obtain a set of enterobacteria, with the strain TSHA-91 being especially effective. It was identified as Klebsiella planticola (Tab. 1).

Tab. 1. Nitrogenase activity strains of genus Klebsiella, isolated from the rhizosplane of cucumber plants

NN strains	Nitrogenase activity (nmol C_2H_4 ml^{-1} 24h^{-1})
Klebsiella planticola TSHA-91	34000
Klebsiella sp. э-9ì	4900
Klebsiela sp. э-10	5130
Klebsiela sp. э-12	16000
Klebsiella sp. э-13	4000

It has been shown recently that favourable effects of diazotrophs on plants depend not only on the nitrogen fixation, but also on synthesis of phytohormones as well as on inhibition of phytopathogenic fungi. We studied biosynthesis of growth factors by Klebsiella planticola in an experiment with water-cress as a test plant. Thirty water-cress seeds were placed on a filter-paper in plates where centrifugate of 24 hours bacterial culture was added. The length of roots and stems of germs was measured in 48 hours. The data obtained are shown in table 2. As can be seen, Klebsiella planticola is able to synthesize growth substances. Under sufficiently high dilution of cell-free bacterial culture (1 by 1000), (only by one thousand) considerable acceleration of growh of both the roots (by 86 percent) and stems (by 80 percent) of water-cress test plant was observed.

The ability of the associative diazotroph to produce antibiotic-like substances was determined by means of agar-diffusion method using cultures of various pathogenic fungi as test object. Table 3. shows that Klebsiella planticola suppressed growth of Penicillium, Aspergillus, Thelaviopsis and also exerted a certain inhibitory action on fungi of genera Alternaria and Mucor. Fungistatic ability of these bacteria is potentially useful for biological protection of hot-house plants.

It is known that availability of a strain of diazotroph having above properties is not sufficient to produce efficient bacterial fertilizers. The microorganism used as a fertilizer must be able to dominate on the roots of a given plant. Predominance of a microorganism on the roots of a plant depends on its adhesion ability, its invasion into plant cells as well as intracellular reproduction (colonization of roots). Therefore, it seemed to be necessary to study the ability of Klebsiella planticola to absorb at the plant roots and colonize them. Experiments with gnotobiotic cultures of lettuce, cabbage oats and pea (Tab. 4) showed that Klebsiella planticola possesses abilities of absorbtion and extracellular invasion into the plants.

Tab. 2. Effect of plant growth regulators, synthesizing Klebsiella planticola TSHA-91 strain on the growth of watercress

Plant organs	Concentration of centrifugate bacterial culture				
	water (control)	without dilution	1:10	1:100	1:1000
Roots	0,58*	0,18	0,25	0,51	1,08
	100**	31	43	88	186
Leaf	0,10	0	0,01	0,05	0,18
	100	0	10	50	180

Note: *) numerator - length in cm
**) denominator - % to control

Tab. 3 Fungistatic activity of Klebsiella planticola TSHA-91 strain

Test - objects	The size of the zone inhibition growth of fungi
Aspergillus flavus	3,1 cm
Aspergillus niger	4,0 cm
Penicillium brevicum compostum	3,5 cm
Fusarium moniliforte	zones of inhibition aren't produced
Fusarium vasinfectum	zones of inhibition aren't produced
Fusarium oxysporum 151	zones of inhibition aren't produced
Alternaria sp.	3,5 cm
Mucor fragilis	3,0 cm
Thelaviopsis basicola	5,5 cm
Diameter of hole for Klebsiella planticola	1,2 cm

Tab. 4 Adhesion-capability and invasion into the plants Klebsiella planticola TSHA-91 strain and its varieties
(pot experiment, 7 days,1991)

NN	NN strains	Plants							
		Lettuce		Cabbage		Pea		Oats	
		root	leaf and stem	root	leaf and stem	root	leaf and stem	root	leaf and stem
1.	TSHA - 91 (initial)	+	+	+	+	+	-	+	+
2.	TSHA/Rif	+	+	+	+	+	-	+	+
3.	TSHA/Rif /p0R95	+	+	+	+	+	-	+	+
4.	TSHA/Rif /pA1z22110	+	+	+	+	+	-	+	-
5.	TSHA/NR/478	+	+	+	+	+	-	+	-
6.	TSHA/M 25 - 8	+	-	+	-	+	-	+	-

Further studies were devoted to persistance of bacteria in rhizoplane of cucumber cultivated in protected ground condition. Klebsiella planticola was investigated in terms of its capacity to be adsorbed of plant roots and to colonize them, and it was found out that bacteria were restricted to roots and were practically absent in the soil (Tab. 5).

Tab. 5 The number of cells Klebsiella planticola TSHA-91 strain in the rhizosplane and the rhizosphere of cucumber plants
(thousands/1kg of soil d.m.)

Variants of experiment	Rhozoplane	Rhizospera
Control (without inoculation)	0	0
With inoculation Klebsiella planticola TSHA - 91	1190	0,05

So, the Klebsiella planticoila ability to penetrate into the root systems permitted to suppose that this microorganism exerts a positive effect on plants as it displays a high nitrogen fixation capacity, and is able to to synthesize growth substances and to inhibit the phytopathogenic fungi.

Inoculum preparation and inoculation of field experiments: Klebsiella planticola cell suspensions were mixed with finely sieved autoclaved sterilized peat, adjusted to pH 6,8 with $CaCO_3$, at a final concentration of about 10^{10} colony forming units per g of peat. The moisture content of the mixture was 40% and the inoculent was stored at room temperature in sterile polythylene bags. At the time of use the peat contained 10^8 cells per g of peat. Control plots in all field experiments were treated with the same amount of sterile peat. Inoculation of cucumber and tomato with Klebsiella planticola was carried out in all experiments three weeks after plant emergence. The experiment was carried out in the green house in the protected ground conditions with 5 randomized blocks containing two treatment plots, each 36 m^2, with one inoculated with the Klebsiella inoculant and the other (non-inoculated) control treated with the same amount of sterile carrier.

Studies of Klebsiella planticola inoculation influence on the yields of vegetables showed a distinct positive effect.

As shown in the table 6, cucumber yield grew by 21-23%, that of tomatoes - 31% (Tab. 7, Fig. 1).

Tab. 6 Effect of inoculation of Klebsiella planticola TSHA-91 strain on Yield cucumber plants (1992)

Date	Without inoculation (kg/m^2)	With inoculation (kg/m^2)	%
February--March	3,04	3,97	30,3
April	4,21	4,73	12,3
May	4,77	5,75	20,6
June	1,88	2,63	39,9
February--June	13,91	16,85	22,7

The yields of potato plants as affected by tuber bacterization with Klebsiella planticola are shown in table 8. Potato yield rose by 52-78%.

The effectiveness of inoculation of potato tubers with Klebsiella planticola under conditions of field experiments are shown in table 9. Potato yield increased by 17-21%.

As shown in table 10, pumpkin yield grew by 31%.

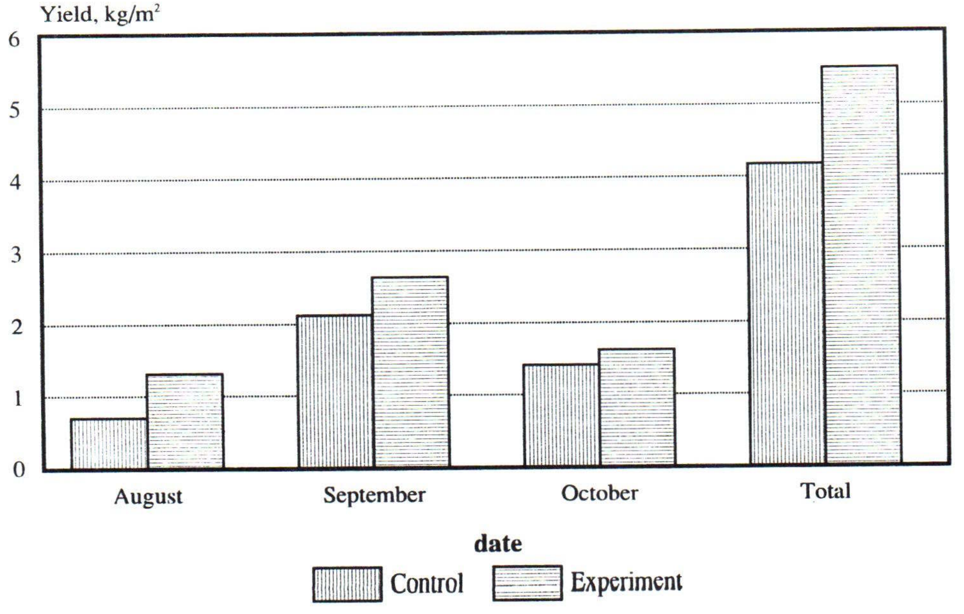
So, noticeable increase in production is explained by Klebsiella planticola ability to permanent reproduction, stable existence and spreading on plant roots during the whole vegetation period, as well as by the root system protection of phytopathogenic fungi. Klebsiella planticola strain is reccommended as a bacterial fertilizer for vegetables, mainly in the greenhouses for obtaining ecologically pure production in conditions of sustainable agriculture.

Tab. 7 Effect of inoculation with Klebsiella planticola TSHA-91 strain on tomato plants (1992) yield

Date	Yiels, kg/m²		gross yield of tomato/kg	
	Control	Experiment	Control	Experiment
August	0,68	1,35	50,11* 100**	9828,8 196
September	2,09	2,60	15194,9 100	18871,2 124
October	1,42	1,55	10355,2 100	11301,6 109
Total August--October	4,19 100	5,50 131	30561,6	40001,6

Note: * numerator - in kilogrammes
** denominator - % to control

Fig. 1 Effect of inoculation with Klebsiella planticola TSHA-91 strain on the yield of tomato plants



Tab. 8 The yield of potato plants as affected by tuber bacteriozation with Klebsiella TSHA-91 strain

NN	Variants of experiment	Yield kg/m^2	Yield % to control
1.	Control (without bacterization)	5,10	100
2.	Bacterization of potato tubers at the time of planting	7,75	152
3.	Bacterization of potato tubers 20 days prior to planting	9,10	178

Tab.9 The effectiveness of inoculation of potatoes tubers with Klebsiella planticola TSHA-91 strain under the conditions of field experiment

Variants of experiment	Total yield in t/ha	% to control the	On fractions, t/ha, size of potato tubers >80g	80 -50g	50-30g	<30g	comodity output	Coefficient of reproduction
Planting of potatoes without inoculation	19,0	100	9,0	3,5	4,0	2,5	16,5	2,01
Planting of potato tubers with inoculation	23,0	121	17,0	2,0	1,5	2,5	20,5	1,00
Potato tubers without planting and without inoculation	23,7	100	17,1	3,5	2,0	1,2	22,5	1,60
Potato tubers without planting but with inoculatic	27,8	117	17,5	5,0	3,0	2,3	25,5	2,28

Tab. 10 Results from comparing different bacterial inoculants for their effects on squash (Cucurbita mixta) and pumpkin (Cucurbita pepo) yields of fruits in 1992

Innoculant	Squash	Pumpkins
	t/ha	
Control	24.1	76.4
Inoc 1 (USA)	24.7	96.1
Inoc 2 (USA)	29.3	74.4
Inoc 3 (USA)	26.5	90.1
Inoc 4 (USA)	27.5	88.3
Inoc 5 (Russia)	27.6	99.8

F test fruits 46.5
F test innoculants 0.85 N.S.
F test fruits x innoculants 0.77 N.S.

REFERENCES

Thomas-Bauzon D., Weinhard P., Villecourt P. and Balandreau J.(1982)- The spermosphere model. 1. Its use in growing, counting and isolation N-fixing bacteria from the rhizosphere of rice. Can. J. Microbiol. 28: 922-928.

Received: 7. 09. 1995.
Accepted: 14. 03. 1996.

ASOCIJATIVNA SIMBIOZA ZEMLJIŠNIH DIAZOTROFNIH BAKTERIJA I BILJAKA KAO I NJEN DOPRINOS PRINOSU POVRTARSKIH USEVA

-originalni naučni rad-

V. T. Emtsev
Odsek za mikrobiologiju, Timirjazev, Poljoprivredna akademija, Moskva

Rezime

U radu je izučavana asocijativna sposobnost diazotrofnih sojeva bakterija, dobijenih metodom analitičke selekcije za permanentnu reprodukciju i razvoj u rizosferi negljivičnih vrsta (pre svega, povrtarskih useva). Osnovni zadatak je bio da dobijeni sojevi bakterija zadrže svoje prvobitne osobine, kao što su azotofiksacija, sinteza fitohormona i produkcija nekih fungistatičkih agenasa, što se u krajnjem pokazalo vrlo pozitivno u smislu povećanja prinosa mnogih povrtarskih kultura.