UDC 575
DOI: 10.2298/GENSR1301237P
*Original scientific paper*

# SELECTION AND RAPD ANALYSIS OF *Pseudomonas ssp.* ISOLATES ABLE TO IMPROVE BIOLOGICAL VIABILITY OF POTATO SEED TUBERS

Dobrivoj POŠTIĆ[1], Mira STAROVIĆ[1], Tatjana POPOVIĆ[1], Predrag BOSNIĆ, Aleksandra STANOJKOVIĆ- SEBIĆ[2], Radmila PIVIĆ[2], Dragana JOŠIĆ[2]

[1]Institute for Plant Protection and Environment, Belgrade,
[2]Institute of Soil Science, Belgrade

Postic D., M. Starovic, T. Popovic, P. Bosnic, A. Stanojkovic-Sebic, R. Pivic, and D. Josic (2013): *Selection and RAPD analysis of Pseudomonas ssp. isolates able to improve biological viability of potato seed tubers.* Genetika, Vol 45, No. 1, 237-249.

Indoleacetic acid (IAA) producing *Pseudomonas* isolates from the rhizosphere of maize (Q4 and Q20), alfalfa (Q1 and Q16) and wild red clover (B25) were selected for the investigation of their effect on the biological vitality of the potato seed tubers. The production of IAA ranged from 4.09 to 15.9 $\mu gmL^{-1}$ after 24h of cultivation and 4.08 to 26.4 $\mu gmL^{-1}$ after 48h of cultivation. The molecular comparison by RAPD analysis also was done. RAPD patterns of selected *Pseudomonas spp.* isolates obtained by BC318, AF14, SPH 1 and AP 10 primers demonstrated the suitability of RAPD method in distinguishing a high variability among the four isolates (44 to 68%). The effect on the biological viability of potato (industrially important variety Pirol) was observed during the seven weeks of sprouting at the temperature of 18-20°C. Potato tubers treated by the selected isolates formed slightly lower number of sprouts, but statistically higher mean length - up to 129.9% higher than the control. The mean sprouting capacity was 64.5% higher than a control. Obtained results suggested positive

***Corresponding author:*** Dobrivoj Poštić, Institute for Plant Protection and Environment, T. Drajzera 9, 11040 Belgrade, Serbia, Tel: +381 11 2663-672; Fax: +381 11 2669-860; e-mail: pdobrivoj@yahoo.com

effects of selected IAA producing *Pseudomonas* isolates on the length of potato tubers and sprouting capacity as the parameters which define biological viability.

*Key words: Pseudomonas* spp., IAA, RAPD, biological viability, potato, tuber, sprout, sprouting capacity

## INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) promotes growth and development of plants (PGRs) through several mechanisms, including the production of secondary metabolites and siderophores; phosphate solubilization; antagonism to soil-borne root pathogens; modification of root morphology which increase root surface area for the uptake of nutrients as well as stress responses (HASS and KEEL, 2003; DWIVEDI and JOHRI, 2003; BANERJEE *et al*., 2006; KOKALIS-BURELLE *et al*., 2006; GOLDSTEIN *et al*., 2007). These bacteria promote plant growth directly through the production of plant hormones - organic substances that influence physiological processes of plants at extremely low concentrations (PERSELLO-CARTIEAUX *et al*., 2003) with a significant regulatory role in plant growth and development (DOBBELAERE *et al*., 2003).

Among the five classes of well-known PGRs (auxins, gibberellins, cytokinins, ethylene and abscisic acid), a greater attention has been given to the role of phytohormone auxin, especially to indole-3-acetic acid (IAA). It has been estimated that 80% of bacteria isolated from the rhizosphere can produce plant growth regulator IAA (PATTEN and GLICK, 2002) as the most common phytohormone. IAA is physiologically most active auxin in plants stimulating cell elongation, cell division and differentiation (HAYAT *et al.,* 2010).

The genus *Pseudomonas* includes some species that live in close association with plants and may be beneficial to the host. PGP effects in different crops were well known, showing that bacterial inoculates are able to stimulate plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from the diseases (LUGTENBERG *et al*., 2002; GHOLAMI *et al*., 2009; JARAK *et al.*, 2012). To compare the *Pseudomonas* isolates and to estimate their diversity, the RAPD method has been chosen as a fast and sensitive method, able to provide characteristic and reproducible fingerprints of complex genomes, without a need for the prior sequence information for different bacterial genera (PICARD *et al*., 2000; RAMESHKUMAR *et al*., 2012).

Biological viability of potato seed tubers, as tuber ability to establish new vegetative shoots after the storage period (POŠTIĆ *et al*., 2010) was estimated on the basis of the size of the sprouting shoot. The term is used to describe the physiological properties of seeds which control their ability to germinate rapidly in the soil (MILOŠEVIĆ *et al*., 2010; POŠTIĆ *et al*., 2011; POŠTIĆ *et al*., 2012). Biological viability of potato tubers is predetermined by the properties of seed tubers: physiological age, rate of sprout development, mass of tuber and general health.

Physiological age refers to the viability of tubers to be used for seed (PAVLISTA, 2004). It is defined as the stage of tuber development (STRUIK, 2007) or the physiological state which affects productivity (BOHL *et al*., 2003) and depends on the variety, growing conditions during the seed tuber formation, maturity at harvesting, storage conditions, the level damage and health conditions. The mass of potato tubers and the rate of sprout development at planting time determine the biological viability of the tubers and can have a strong effect on the speed of shoot emergence, stem growth and, ultimately, the crop yield (STRUIK, 2007). POŠTIĆ *et al*., (2011) established that only completely healthy seed tubers can have high bilogical viability.

Assessment of the biological viability through these parameters is a very important indicator of the final yield, measured as an estimate of the number of plants per area, or the size/mass of the tubers (STURZ *et al*., 2000). This is particularly significant for the varieties such as Pirol, which is used on an industrial scale in the production of crisps.

In this work, the indigenous *Pseudomonas* isolates, able to produce IAA, were selected to investigate their effects on the potato seeds tubers (Pirol). RAPD analysis was used for the molecular comparison of selected isolates.

## MATERIALS AND METHODS

**Bacterial strains.** *Pseudomonas* isolates were isolated from the rhizosphere of different plants: maize (Q4 and Q20), alfalfa (Q1 and Q16) from pseudogley soil in Sumadija and from the rhizosphere of wild red clover (B25) growing in polluted soil in Pancevo, Serbia. Bacteria were grown in King B medium at standard conditions (at 26$^{o}$C for 24h).

**Production of IAA.** A standard procedure and standardized bacterial suspensions ($OD_{600}$ of 0,625) were used for IAA production assay (GLICKMANN and DESSAUX, 1995). The King B medium, containing different concentration of tryptophan (0; 2.5 and 5 mM) was inoculated with 10µl of bacterial culture and incubated at 26$^{o}$C for 24h and 48h. To standardize the concentration of isolates ($OD_{600}$ = 1) the absorbance at 600 nm was measured. The obtained supernatant of appropriate concentration of *Pseudomonas* isolates ($OD_{600}$ = 1) was mixed with Salkowski reagent (2% 0.5M ferric chloride in 35% perchloric acid) (1:2 v/v). To obtain the quantitative estimation of IAA production and construct the IAA standard curve, the intensity of pink color at 530 nm was measured.

**RAPD**. Genomic DNA of the rhizospheric *Pseudomonas* was extracted from 200 µl cultures using the protocol described earlier (ROSS *et al*., 2000). PCR was carried out with DreamTaqGreen Master Mix (ThermoScientific Fermentas), 25 ng of total bacterial DNA as template, 100pM of appropriate primer and the following amplification program: 5 min at 95°C for initial denaturation, (1 min at 95°C, 1 min at 37°C, 2 min at 72°C) for 35 cycles and 7 min at 72°C for final extension. List of primers are given in Table 1.

*Table 1. Primers used in RAPD analysis*

| Primer | Sequence (5'–3') | Reference |
|---|---|---|
| SPH 1 | GACGACGACGACGAC | DOOLEY *et al*., 1993 |
| AP 10 | CAGGCCCTTC | SELENSKA-POBEL *et al*., 1996 |
| BC318 | CGGAGAGCGA | MLIKI *et al*., 2001 |
| AF14 | GGTGCGCACT | MLIKI *et al*., 2001 |

**Measuring sprouting capacity**. The Pirol variety of potato was selected as it is an early variety, commonly used for the production of crisps. Samples of 30 tubers weighing 80g each were treated by the *Pseudomonas sp*. isolates in two concentrations ($10^7$ and $10^8$ CFUml$^{-1}$). Using the sprouting method, the indicators of biological viability were investigated over the seven weeks. The samples were exposed to the following conditions for the first three weeks: temperature of 18-20°C, relative humidity of 90-95%, and without light; and to different conditions for the following 4 weeks: (18-20°C, RH=75% and light of 40-65W/4-5m² 9h of light during the day /24h.

The biological viability of tubers was measured every week from the start of sprouting. The sprouts equal or longer than 3mm were taken into account. The mean number of sprouts per tuber was calculated from all 30 tubers sampled and the length of the sprout was determined by measuring the longest sprout on each tuber to the nearest 1 mm using calliper.

The sprouting capacity (%) was determined at the end of sprouting by the method of STRUIK and WIERSEMA, (1999). Sprouts were removed from all 30 tubers and their mass measured. The percentage sprouting capacity has been calculated by dividing the mass of sprouts by the mass of tubers.

The obtained experimental data were processed by a mathematical statistical procedure using the statistical package STATISTICA 8.0 for Windows (Analytical software, Faculty of Agriculture, Novi Sad, Serbia). The differences between the treatments were determined by analysis of the variance (ANOVA) and the least significant difference test (LSD) was used for the individual comparisons.

## RESULTS AND DISCUSSION

**IAA production**. Production of IAA in KB medium without the addition of tryptophan and the effect of two tryptophan concentrations on the tested isolates are shown in Figure 1. The differences among the isolates of *Pseudomonas* spp. in their ability to produce IAA were previously reported (AHMAD *et al*., 2005; JOSIC *et al*., 2012b). *Pseudomonas* isolates in our investigation have also showed the differences in ability to produce IAA. Selected isolates produced 4.09 to 15.9 µgmL$^{-1}$ of IAA after 24h of cultivation and 4.08 to 26.4 µgmL$^{-1}$ after 48h of cultivation. RAMEZANPOUR *et al*. (2011) reported IAA production for more than 100 rhizospheric isolates able to produce IAA ranging from 17.7 to 95.9 mg L$^{-1}$ with an average of 39.8 mg L$^{-1}$. Quantities of about 40.7 mgL$^{-1}$ of IAA were reported for *P. aeruginosa*, *P. putida* and *P. fluorescens*. One strain (*P. putida* MZ15) produced the highest amount of IAA (95.9 mgL$^{-1}$). These authors reported that the average production of IAA by different *Pseudomonas* species was very close to each other. In comparing to these results, the selected isolates in our study showed a high level of IAA production.

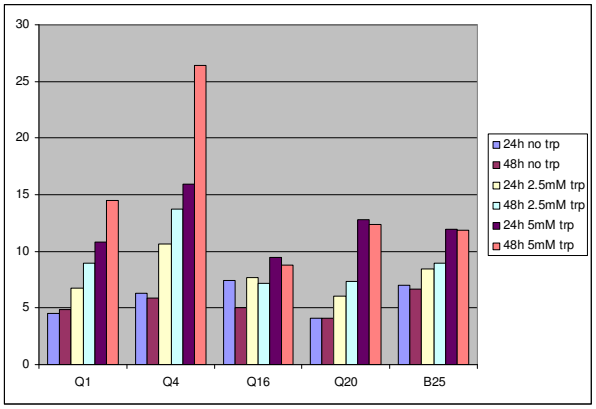


Figure 1. IAA production by selected *Pseudomonas* spp. isolates in different conditions

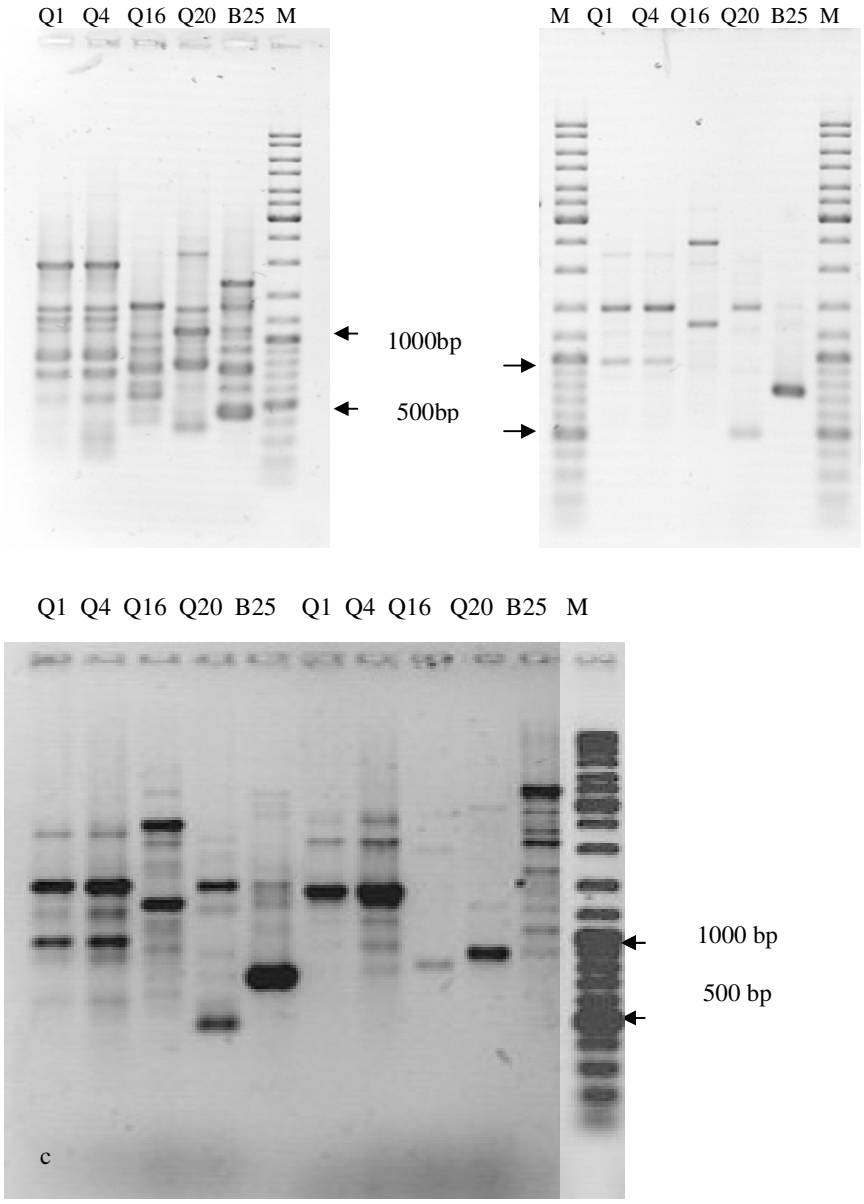


Figure 2. RAPD analysis of IAA producing Pseudomonas isolates. Patterns were obtained by a) SPH 1 primer; b) AP10 primer; c) BC318: lane 1-5 and AF14: lane 6-10. M-marker

**RAPD**. Based on RAPD method, the genotyping of *P. fluorescens* isolates producing antifungal compounds were carried out. PICARD *et al*. (2000) differentiated 64 genotypes from phlD-containing *Pseudomonas* isolated from maize rhizosphere. The results of this study showed the possibility of using RAPD method to distinguish between variability among the selected *Pseudomonas* isolates. RAPD patterns revealed DNA bands ranging from 100 to 2500 bp and clear variations in the PCR products were observed between the isolates (Figure 2). The amplified products obtained by SPH1 primer produced clear and distinct polymorphic bands in greater numbers in comparison to the other RAPD primers. The similarity levels of all obtained RAPD patterns were shown in figure 3.

RAMESHKUMAR *et al*. (2002) also used RAPD analysis for differentiation of 40 strains from the rhizosphere of rice and sugarcane, exhibiting plant growth promotion and antifungal activity, because of the limitation of other morphological and biochemical methods. Characterization by PCR-RAPD analysis and biochemical methods was reported by PRASANNA REDDY and RAO (2009) for ten isolates belonging to fluorescent *Pseudomonas* selected from rice rhizosphere which exhibited strong antifungal activity against *P. oryzae* and *R. solani*. The differentiation of the isolates belonging to *P. plecoglossicida, P. fluorescens, P. libaniensis,* and *P. aeruginosa* was achieved through several genomic DNA fingerprinting techniques: RAPD, amplified ribosomal DNA restriction analysis (ARDRA) and rep-PCR (RAMESHKUMAR *et al*., 2012). Also, rep-PCR is applied to estimate the diversity of *Pseudomonas* isolates from chernozem in Vojvodina (JOŠIĆ *et al*., 2012a) and *Pseudomonas* isolates from pseudoglay soil in Serbia (JOSIC *et al*., 2012b).

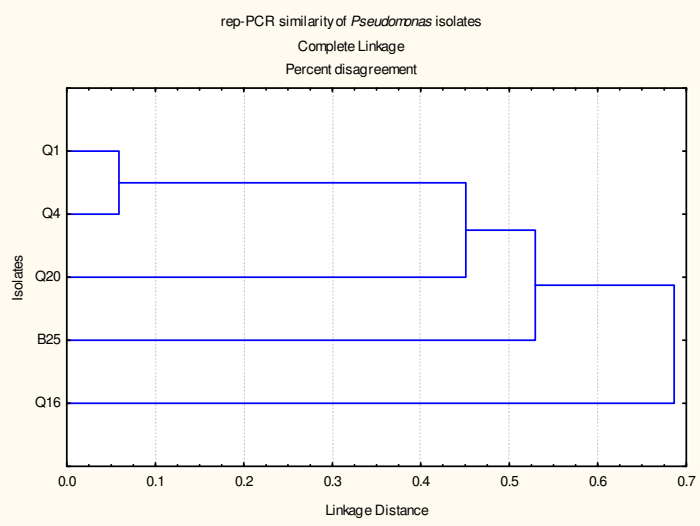


Figure 3. The similarity of selected *Pseudomonas* spp. isolates on the basis of RAPD analysis using four primers

**Sprouting capacity.** It has been reported that seed treatment with PGP *Pseudomonas* strains improved seed germination, seedling vigor, seedling emergence for pearl millet (NIRANJAN *et al*., 2004). SHAUKAT *et al*. (2006a; 2006b) found that in wheat and sunflower some PGPR caused increase in seed germination, and, in some cases, achieving increases up to 100% greater than controls, and may have occurred by a better synthesis of auxins (BHARATHI *et al*., 2004). The effect on seed germination, seedling growth and yield of field grown maize, caused by *P. fluorescence* and *P. putida* and others PGPR, were evaluated by GHOLAMI *et al*. (2009). They showed that bacterial treatments had a more stimulating effect on growth and development of plants in non-sterile than sterile soil.

Considering that the extracellular metabolites of *Pseudomonas* isolates usually contain many active substances, including IAA, we investigated the effects of IAA producing *Pseudomonas* isolates on potato sprouting capacity.

The function of the plant regulator IAA in the control of dormancy and sprouting of the tubers is not completely established. It is reported that the content of free and multiple IAA in the tubers is the highest at the start of tuber dormancy (SORCE *et al*., 2009), while the concentration of free IAA was low at the start of sprouting. However, IAA was found not to be directly responsible for the inhibition of tuber sprouting (SORCE *et al*., 2000; 2009). PAVLISTA (2004) reported that IAA directly involved in controlling, the phenomenon known as the apical dominance in tubers.

The number of sprouts per tuber has increased until the third week and then stayed unchanged until the end of sprouting (Table 2). Bacterial isolates of *Pseudomonas sp*. Q1, Q4 and Q20 in both concentrations as well as Q16 in concentration of $10^8$ CFUml$^{-1}$ stimulated tuber sprouting and increased the number of sprouts per tuber compared to control in the first week However, in the second and third week, only the tubers treated by the isolate Q20 in concentration of $10^7$CFUml$^{-1}$ produced more sprouts than the control. The treatments with isolate B25 caused the smallest mean number of sprouts per tuber.

The tubers treated with all selected *Pseudomonas* formed significantly longer sprouts ranging from 10.3 to 129.9% in relation to the control and the growth rate varied from week to week (Table 3). After seven weeks of sprouting, the minimum mean length of sprouts per tuber was 23.4 mm, found in the control. The largest mean lenght was 53.8 mm per tuber and this was measured on the variety treated with the bacterial isolate Q1 in concentration of $10^8$ CFUml$^{-1}$. Variance analysis established the importance of the following factors on the number and length of sprouts: bacterial isolate in both concentrations $10^7$ and $10^8$ CFUml$^{-1}$ (A) and the duration of sprouting (B). The AxB interaction had a significant effect only on the length of sprouts in concentration $10^8$ CFUml$^{-1}$, but not the number of sprouts per tuber or in the concentration of $10^7$. The length of sprouts per tuber also increased with the duration of sprouting, which matched with results obtained by GACHANGO *et al*. (2008) and POŠTIĆ *et al*. (2010; 2012). The increase of the heat accumulation, together with the duration of sprouting, have caused the formation of longer sprouts which support the results obtained earlier (GACHANGO *et al*., 2008; POŠTIĆ *et al*., 2010; RYKACZEWSKA, 2010; POŠTIĆ *et al*., 2012).

The tubers treated by the selected *Pseudomonas* isolates showed higher sprouting capacity (between 18.1 and 64.9%) in relation to the control (Table 4). Bacterial isolates Q1, Q20 and B25 showed higher sprouting capacity in concentration of $10^8$ CFUmL$^{-1}$, since isolate Q4 showed higher capacity if the concentration of $10^7$ CFUmL$^{-1}$ was applied. Isolate Q16 increased sprouting capacity around 30% in both applied concentration.

*Table 2. The number of sprouts per tuber by the isolate concentration and duration of sprouting*

| | No of sprouts per tuber | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolates | Q1 | | Q4 | | Q16 | | Q20 | | B25 | | K |
| | concentrations | | | | | | | | | | |
| Time (week) | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | |
| I | 4.70 | 4.77 | 4.32 | 4.72 | 3.67 | 4.50 | 5.60 | 4.87 | 3.50 | 3.65 | 3.92 |
| II | 5.12 | 5.20 | 5.08 | 5.05 | 4.83 | 4.50 | 6.10 | 5.12 | 3.87 | 3.78 | 5.22 |
| III | 5.31 | 5.48 | 5.32 | 5.27 | 5.00 | 5.62 | 6.15 | 5.60 | 4.25 | 4.53 | 5.72 |
| Index | 92.83 | 95.80 | 93.01 | 92.13 | 87.41 | 97.74 | 106.96 | 97.90 | 74.30 | 78.78 | 100 |

| Analysis of variance | Bacterial isolate (A) | | Duration of sprouting (B) | | A x B | |
|---|---|---|---|---|---|---|
| | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ |
| $LSD_{0.05}$ | 0.47 | 0.69 | 0.33 | 0.49 | 0.82 | 1.19 |
| $LSD_{0.01}$ | 0.65 | 0.95 | 0.46 | 0.67 | 1.12 | 1.64 |

*Table 3. The length of sprouts per tuber by the bacterial isolates and the duration of sprouting*

| | The length of the longest sprout per tuber | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolates | Q1 | | Q4 | | Q16 | | Q20 | | B25 | | K |
| | Concentration | | | | | | | | | | |
| Time (week) | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | |
| I | 8.4 | 9.2 | 8.2 | 8.6 | 9.3 | 8.4 | 8.7 | 7.3 | 6.5 | 9.1 | 8.1 |
| II | 13.4 | 12.5 | 11.0 | 11.5 | 12.2 | 11.3 | 11.9 | 10.8 | 8.9 | 12.0 | 11.4 |
| III | 14.7 | 13.8 | 14.2 | 13.8 | 12.9 | 13.2 | 14.1 | 13.2 | 12.2 | 13.2 | 12.3 |
| IV | 18.0 | 16.0 | 16.2 | 17.7 | 14.1 | 16.6 | 15.8 | 16.5 | 14.9 | 14.3 | 12.7 |
| V | 18.3 | 22.4 | 18.7 | 29.5 | 17.8 | 25.1 | 21.7 | 20.6 | 21.2 | 22.3 | 15.6 |
| VI | 18.7 | 33.6 | 22.1 | 42.5 | 20.2 | 33.4 | 29.6 | 29.8 | 28.3 | 32.4 | 19.6 |
| VII | 25.8 | 53.8 | 26.8 | 52.3 | 25.4 | 39.8 | 38.1 | 39.2 | 32.6 | 37.2 | 23.4 |
| Index | 110.3 | 229.9 | 114.5 | 223.5 | 108.6 | 170.1 | 162.8 | 167.5 | 139.3 | 159.0 | 100 |

| Analysis of variance | Bacterial isolate (A) | | Length of sprouting (B) | | A x B | |
|---|---|---|---|---|---|---|
| | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ |
| $LSD_{0.05}$ | 1.39 | 2.47 | 1.51 | 2.67 | 3.69 | 6.53 |
| $LSD_{0.01}$ | 1.91 | 3.39 | 2.07 | 3.66 | 5.06 | 8.96 |

*Table 4. Sprouting capacity (%) by bacterial isolates and the duration of sprouting*

| | Sprouting capacity (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolates | Q1 | | Q4 | | Q16 | | Q20 | | B25 | | K |
| Conc. | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | $10^7$ | $10^8$ | |
| Mean | 3.91 | 5.45 | 5.43 | 4.71 | 4,37 | 4,33 | 4,58 | 5,31 | 3,96 | 4,29 | 3,31 |
| Index | 118.1 | 164.6 | 164.1 | 142.3 | 132.0 | 130.8 | 138.4 | 160.5 | 119.6 | 129.6 | 100 |

A significant correlation was established between physiological age and sprouting capacity. Physiological age determines the behavior of each bud on the seed tuber and thus affects the number of the sprouts per eye (STRUIK, 2007; POŠTIĆ *et al*., 2010; 2012). The previously gained results showed that the number of sprouts per tuber (POŠTIĆ *et al*., 2010) and the length of sprouts per tuber were increasing with the increase of physiological age of seed tubers (POŠTIĆ *et al*., 2010; 2012). The increase of the heat accumulation with duration of sprouting have caused the formation of longer sprouts in control potato seed, which match with the results obtained earlier (GACHANGO *et al*., 2008; POŠTIĆ *et al*., 2010; RYKACZEWSKA, 2010; POŠTIĆ *et al*., 2012). Accumulation of heat during sprouting period significantly increased the sprouts length on all samples treated with *Pseudomonas* isolates, especially with Q1 and Q4 (129.9% and 123.3%). These results may be explained by low decreasing of sprouts number caused by *Pseudomonas* application of and stimulating of the main sprouts.

The selected IAA producing, bacterial isolates showed a positive effect on the sprouting capacity. The accumulation of heat in the tubers had a positive effect on the sprouting as it increased the speed of the physiological processes and ageing. The secondary metabolites of applied *Pseudomonas* isolates, including IAA, caused stimulation on sprouts length and vigor, and increasing of sprouting capacity. The isolates Q1 and Q4, that belong to the same RAPD group, and isolate Q20 showed higher (160%) sprouting capacity than other applied *Pseudomonas* isolates.

## CONCLUSION

A selection of indigenous *Pseudomonas* isolates was conducted on the basis of IAA production, which ranged from 4.08 to 26.4 $\mu g mL^{-1}$. RAPD method revealed a low variability between isolates Q1 and Q4, and high variability among these and others applied isolates (44 to 68%) when BC318, AF14, SPH 1 and AP 10 primers were applied. Statistically significant differences were shown in the sprouts length per tuber and the biological viability of the tubers, depending on the *Pseudomonas* isolate and the duration of sprouting. The *Pseudomonas* isolates Q1, Q4 and Q20 caused the sprouting capacity about 60% higher than control. The importance of higher sprouting capacity is especially useful for short season, and an early potato production with lower number of primary stems. This would lead to faster formation of heavier tubers, and a higher yield which is highly desirable in commercial cultivation of potatoes.

ACKNOWLEDGEMENT

Project No. III46007 is funded by the Ministry of Education and Science, Republic of Serbia.

Received July17h, 2012

Accepted March 08th, 2013

REFERENCES

AHMAD, F., AHMAD, L., M.S. KHAN (2005): Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas*in presence and absence of tryptophan. Turk. J. Biol., *29*: 29-34.

BANERJEE, M.R., YESMIN, L., J.K. VESSEY (2006): Plant growth promoting rhizobacteria as biofertilizers and biopesticides. In: Rai MK (ed) Handbook of microbial biofertilizers. Haworth Press, New York

BHARATHI, R., R.VIVEKANANTHAN, S. HARISH, A. RAMANATHAN, R. SAMIYAPPAN (2004): Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Protec.. *23*: 835–843.

BOHL, W.H., OLSEN, N., LOVE, S.L., P. NOLTE (2003): Seed and planting management. In Potato Production Systems. Publ. Univ. Idaho Extension Chap. *7*: 91-114.

DOBBELAERE, S., VANDERLEYDEN, J., Y. OKON (2003): Plant growthpromoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci. *22*:107–149

DOOLEY, J. J., HARRISON, S.P., MYTTON, L.R., DYE, M., CRESSWELL, A., SKOT, L. J.R. BEECHING (1993): Phylogenetic grouping and identification of *Rhizobium* isolates on the basis of random amplified polymorphic DNA profiles. Can. J. Microbiol. *39*:665-73.

DWIVEDI, D., B.N. JOHRI (2003): Antifungals from fluorescent pseudomonads: biosynthesis and regulation. Curr Sci. *12*:1693–1703

GACHANGO, E., SHIBAIRO, S., KABIRA, J., CHEMININWA, G., P. DEMO (2008): Effects of light intensity on quality of potato seed tubers. African Journal of Agricaltural Research. *3* (10): 732-739

GHOLAMI, A., SHAHSAVANI, S., S. NEZARAT (2009): The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int J Bio Life Sci. *1*(1): 35-38

GLICKMANN, E., Y. DESSAUX (1995): A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl. Environ. Microbiol. *61*: 793-796.

GOLDSTEIN, A.H. (2007): Future trends in research on microbial phosphate solubilization: one hundred years of insolubility. In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Dordrecht, pp 91–96

HAYAT, R., ALI, S., AMARA, U., KHALID, R., I. AHMED (2010): Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. *60* (4): 579-598

HASS, D., C. KEEL (2003): Regulation of antibiotic production in root colonizing Pseudomonas sp. and relevance for biological control of plant disease. Annu Rev Phytopathol. *41*:117–153

JARAK, M., MRKOVACKI, N., BJELIC, D., JOSIC, D., HAJNAL-JAFARI T., D. STAMENOV. (2012): Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. Afr. J. Microbiol. Res. *6*(27): 5683-5690

JOŠIĆ, D., PIVIĆ, R., MILADINOVIĆ, M., STAROVIĆ, M., PAVLOVIĆ, S., ĐURIĆ, S., M. JARAK. (2012a): Antifungal activity and genetic diversity of selected *Pseudomonas* spp. from maize rhizosphere in Vojvodina. Genetika, Belgrade, *44* (2): 377-388

JOSIC, D., DELIC, D., RASULIC, N., STAJKOVIC, O., KUZMANOVIC, D., STANOJKOVIC, A., R. PIVIC. (2012b): Indigenous Pseudomonads from Rhizosphere of Maize grown on Pseudogley Soil in Serbia. Bulgarian Journal of Agricultural Science. *18* (2): 197-206

KOKALIS-BURELLE, N., KLOEPPER, J.W., M.S. REDDY (2006): Plant growthpromoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl Soil Ecol. *31*(1–2): 91–100

LUGTENBERG B., T. CHIN-A-WOENG, G. BLOEMBERG (2002): Microbe–plant interactions: principles and mechanisms. Antonie van Leeuwenhoek. *81*: 373–383.

MILOŠEVIĆ, M., VUJAKOVIĆ, M., Đ. KARAGIĆ (2010): Vigour tests as indicators of seed viability. Genetika. *42* (1): 103-118.

MLIKI, A., STAUB E. J., ZHANGYONG S., G. ABDELWAHED (2001): Genetic diversity in melon (Cucumis melo L.): An evaluation of African germplasm. Genetic Resources and Crop Evolution. *48*: 587-597.

NIRANJAN, S.R., N.P. SHETTY, H.S. SHETTY (2004): Seed bio-priming with Pseudomonas fluorescens isolates enhances growth of pearl millet plants and induces resistance against downy mildew.J.pest.manage. *50*(1):41-48.

PATTEN, C.L., B.R. GLICK (2002): Role of Pseudomonas putida indole-acetic acid in development of the host plant root system. Appl Environ Microbiol. *68*: 3795–3801

PAVLISTA, A. D. (2004): Physiological aging seed tubers. Potato eyes, University of Nebraska. NPE. *16*(1): 1-3.

PERSELLO-CARTIEAUX, F., NUSSAUME, L., C. ROBAGLIA (2003): Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell Environ. *26*:189–199

PICARD, C., F. DI CELLO, M. VENTURA, R. FANI, A. GUCKERT (2000): Frequency and biodiversity of 2,4-diacetylphloroglucinolproducing bacteria isolated from the maize rhizosphere at different stages of plant growth. Appl. Environ. Microbiol. *66*:948-955.

POŠTIĆ, D., MOMIROVIĆ, N., BROĆIĆ, Z., DOLIJANOVIĆ, Ž., ALEKSIĆ, G., TRKULJA, N., Ž. IVANOVIĆ (2010): Effect conditions of production on quality potato seed tubers cv. Desiree. 3rd international scientific/profesional conference, Vukovar, 215-220.

POŠTIĆ, D., MOMIROVIĆ, N., BROĆIĆ, Z., DOLIJANOVIĆ, Ž., ALEKSIĆ, G., Ž. IVANOVIĆ (2011): Assessing the quality of seed potatoes. Proceedings, 46th Croatian and 6th International Symposium on Agriculture, Opatija, 477-480.

POŠTIĆ, D., MOMIROVIĆ, N., BROĆIĆ, Z., DOLIJANOVIĆ, Ž., G. ALEKSIĆ (2012): The evaluation of biological viability of potato seed tubers grown at different altitudes. African Journal of Agricultural Research. *7*(20), 3073-3080.

PRASANNA R.B., K.S. RAO (2009): Biochemical and PCR-RAPD characterization of Pseudomonas fluorescens produced antifungal compounds inhibit the rice fungal pathogens in vitro. EJEAFChe. *8*(10): 1062-1067

RAMESHKUMAR, N., THIRUMALAI ARASU, V., P. GUNASEKARAN (2002): Genotyping of antifungal compounds producing plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* CURRENT SCIENCE. *82* (12):1463-1466

RAMESHKUMAR, N., AYYADURAI, N., KAYALVIZHI, N., P. GUNASEKARAN (2012): Genotypic and Phenotypic Diversity of PGPR Fluorescent Pseudomonads Isolated from the Rhizosphere of Sugarcane (Saccharum officinarum L.) J. Microbiol. Biotechnol. *22*(1):13–24

RAMEZANPOUR, M.R., POPOV, Y., KHAVAZI, K., H.A. RAHMANI (2011): Molecular genosystematic and physiological characteristics of fluorescent pseudomonads isolated from the rice rhizosphere of Iranian paddy fields. African Journal of Agricultural Research. *6*(1):145-151

ROSS, L.I., ALAMI, Y., HARVEY, R.P., ACHOUAK, W., M.H. RYDER. (2000): Genetic Diversity and Biological Control Activity of Novel Species of Closely Related Pseudomonads Isolated from Wheat Field Soils in South Australia. Appl. Environ. Microbiol. 66:1609-1616.

RYKACZEWSKA, K., (2010): The effect of high temperature during storage on the Vigour of Potato Mother tubers. Proceedings of the International Symposium on Agronomy and Physiology of Potato, Turkey, 144-147.

SELENSKA-POBELL, S., EVGUENIEVA-HACKENBERG, E., RADEVA, G., A. SQUARTINI (1996): Characterization of *Rhizobium 'hedusari'* by RFLP analysis of PCR amplified rDNA and by genomic PCR fingerprinting. J. Appl. Bacteriol. *80*:517-28

SHAUKAT, K., S. AFFRASAYAB, S. HASNAIN (2006a): Growth responses of Helianthus annus to plant growth promoting rhizobacteria used as a biofertilizer. J.Agri.Res. *1*(6):573-581.

SHAUKAT, K., S. AFFRASAYAB, S.HASNAIN (2006b): Growth responses of Triticum aestivum to plant growth promoting rhizobacteria used as a biofertilizer. Res. J. Microbiol. *1*(4):330-338.

SORCE, C., LOMBARDI, L., GIORGETTI, L., PARISI, B., RANALLI, P., R. LORENZI (2009): Indoleacetic acid concentration and metabolism changes during bud develoment in tubers of two potato (*Solanum tuberosum* L.) cultivars. Journal of Plant Physiolgy. *166*(10):1023-1033.

SORCE, C., LORENZI, R., CECCARELLI, N., P. RANALLI (2000): Changes in free and conjugated IAA during dormancy and sprouting of potato tubers. Australian Journal of Plant Physiolgy. *27*(4): 371-377

STRUIK, P.C., S.G. WIRSEMA (1999): Seed Potato Technology. Wageningen Academic Publishers, Netherland, 1-383

STRUIK, P.C. (2007): Physiological Age of Seed Tubers. Potato Research. *50*: 375-377

STURZ, A.V., W. ARSENAULT, B. SANDERSON (2000): Production of Processing Potatoes from Whole Seed. Agriculture, Fisheries and Aquaculture. P. E. Island, Canada. 1-3.

WANG, Q., E. TANNE, A. ARAV and R. GAFNY (2000): Cryopreservation of *in vitro*-grown shoot tips of grapevine by encapsulation-dehydration. Plant Cell Tissue and Organ Culture, *63*: 41–46.

# SELEKCIJA I RAPD ANALIZA IZOLATA *Pseudomonas* spp. KOJI POBOLJŠAVAJU BIOLOŠKU SPOSOBNOST KRTOLA KROMPIRA

Dobrivoj POŠTIĆ[1], Mira STAROVIĆ[1], Tatjana POPOVIĆ[1], Predrag BOSNIĆ, Aleksandra STANOJKOVIĆ- SEBIĆ[2], Radmila PIVIĆ[2], Dragana JOŠIĆ[2]

[1]Institut za zaštitut bilja i spoljašnje sredine, Beograd,
[2]Institut za zemljiste, Beograd

Izvod

Izolati *Pseudomonas* iz rizosfere kukuruza (Q4 i Q20), lucerke (Q1 i Q16) i divlje bele deteline (B25) selektovani su na osnovu produkcije indolsirćetne kiseline (IAA) radi ispitivanja efekta na biološku sposobnosti semenskih krtola krompira. Produkcija IAA iznosila je 4.09 do 15.9 $\mu gmL^{-1}$ posle 24h i 4.08 do 26.4 $\mu gmL^{-1}$ posle 48h kultivacije. Upoređivanje izolata izvršeno je na osnovu RAPD analize. Na osnovu RAPD profila selektovanih *Pseudomonas* spp. izolata, dobijenih amplifikacijom BC318, AF14, SPH1 i AP10 prajmerima, ustanovljen je visok stepen različitosti između 4 izolata (44 do 68%), što je potvrdilo efikasnost RAPD metode pri upoređivanju izolata. Efekat na biološku sposobnosti semenskih krtola krompira (industrijski važnu sortu Pirol) ispitivan je tokom sedam nedelja naklijavanja na temperaturi 18-20°C. Krtole tretirane selektovanim izolatima bakterija formirale su nešto manji broj klica, ali je dužina klica statistički značajno veća i do 129,9% u odnosu na kontrolu. Ostvaren je kapacitet klijanja veći do 64,6% u odnosu na kontrolu. Dobijeni rezultati ukazuju na pozitivan efekat selektovanih *Pseudomonas* izolata koji produkuju IAA na dužinu klica i kapacitet klijanja kao parametara koji određuju biološku sposobnosti semenskih krtola krompira.

Primljeno 17. VII. 2012.
Odobreno 08. III. 2013.