Pseudomonas sp. IN TOMATO RHIZOSPHERE (*Lycopersicon esculentum* Mill.)

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ABSTRACT: The aim of research is determining the presence and number of Pseudomonas sp. in tomato rhizosphere. The tests were conducted on a tomato hybrid Big Beef, grown on the soil type pseudoclay-hard. Bacteria isolation was conducted in the root zone 20-40 cm. Rhizosphere was separated mechanically on: rhizospheral soil, root (unsterilized) and root (sterilized). Tripofan Medium was used for isolation of Pseudomonas sp. from the soil. In the obtained results biochemical and morphological characteristics were conducted. Results analysis was conducted with suitable statistical methods. Based on the results, we can conclude that the number of bacteria in different variants is statistically extremely significantly different on Tripofan Medium and statistically significantly different on Nutritious agar.

Key words: *Pseudomonas* sp., tomato, rhizosphere.

INTRODUCTION

Lately, increasing importance is put on the greenhouse tomato production, since all the conditions which tomato requires can be controlled in these facilities (humidity, temperature, soil, etc.). highly fertile, structural soil, with adequate pH value and characterized by great microbiological activity is used in greenhouses. The soil in greenhouses is an environment in which specifi, as well as unspecivid microorganisms exist and different physiological and systematic groups of microorganisms are present. Plant species also have influence on the presence of microorganisms in the soil, primarily through root extracted substances. The aim of this work is the study of *Pseudomonas sp*. in tomato rhizosphere.

Rhizosphere presents a dynamic system and is characterized by its physical, chemical and bilogical characteristics. The biological component of rhizosphere sre plant root and rhizosphere microorganisms which inhabit the soil which is under direct influence of root extracted substances.

Since the rhizosphere zone is rich with root extracted substances or exhudates, as well as nutritious substances which come from the soil, it contains a great number of soil microorganisms, primarily bacteria, it finds favorable conditions in it for its life and activity, so that rhizosphere belongs to the most inhabited bioshperes.

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Literature states that the number of microorganisms in rhizosphere is 10, even 100 times greater than in the surrounding soil. The number of bacteria is the greatest in zone 0-1 cm from the root-exorhizorphere, while at the distance of 15-20 cm from the plant root the number of bacteria decreases even 10 times.(*Paul & Clark,1988*).

In the wheat rhizosphere species from families *Enterobacteriaceae* and *Pseudomonace* show great representation and nitrogenous activity. For triticale, wheat and oats large number of *Pseudomonas sp*. and enterobacteria was determined (*Raičević et al., 1999*) not only in the root zone, but in the root itself. Some of the representatives of *Pseudomonas sp.* can be pathogenous and cause health problems even to people, which is of special interest for the tomato fruit which is used in human nutrition without previous thermal processing.

From the surface of tomato root (*Yoshitaka et al., 1999*) different gram-positive and gram-negative bacteria were isolated which belong to the strains *Burkholderia sp., Xanthomonas sp., Pseudomonas sp., Staphylococcus sp. and Bacillus sp*. These bacteria contribute to the humification of fresh organic substance in the greenhouse soil, but they also contribute to the protection from phytopathogenous bacteria. Bacteria from *Pseudomonas sp.* strain produce antibiotics and so have effect on other rhizosphere bacteria.

Tomato rhizosphere has not been studied well enough which especially relates to the greenhouse tomato.

MATERIAL AND METHODS

The samples of soil were taken in the greenhouse (place Vreoci). Affiliation and number of rhizosphere bacteria, as well as their biochemical characteristics were determined. The soil type is pseudoclay-hard, and tomato is hybrid (Big Beef).

The following were extracted from the samples:

- rhizosphere soil – **varijant II**
- root (unsterilized) – **varijant III**
- root (sterilized) – **varijant IV**

Root sterilization was perfored with 0.5% Na-laurile-sarcosin and 1% NaHCl according to standard methodology.

Since the tomato root is most active in the zone 20-40 cm, that part of the root was determined for isolation of *Pseudomonas sp*. in all three variants. The material (samples) was planted in 3 repetitions, and the number of microorganisms was calculated at 1 g of absolutely dry soil. Selective and enriched nutritious foundation, Tripofan medium, was used.

The results were processed through descriptive and analytical statistics. Main statistic indices were calculated: arithmetic mean, variation interval, standard deviation, standard error and variation coefficient.

Results analysis was conducted: with Bartlett test, variance analysis method (ANOVA), least significant difference test (LSD) and t-test for independent samples.

RESULTS AND DISCUSSION

Determined values of variation coefficient (c_v<30,00%) and graphic presentation show that the data in samples are homogenous, and Bartlett test results (for the samples

on foundation Triptofan Medium $\chi^2 = 0{,}227$, and for the samples of foundation Nutritious Agar $\chi^2 = 1{,}647$) that the tested samples variances are homogenous, which means that the conditions for the use of variance analysis parametric method have been met.

Table 1. Main statistical indices of the number of Pseudomonas sp. bacteria strain isolated on the foundation Triptofan Medium in (000)

Variants	Arithmetic mean	Variation interval	Standard deviation (SD)	Standardna error (SE)	Variation coefficient
II	311,33	281-362	44,16	25,50	14,18
III	625,00	576-681	52,85	30,51	8,46
IV	370,33	329-397	36,30	20,95	9,80

Based on ANOVA results (F=41,264 and F=10,428) we can conclude that the number of bacteria isolated from the soil with different variants statistically differs very significantly on the foundation Triptofan Medium, and statistically differs significantly on the foundation Nutritious Agar. Only the results of LSD-test ($lsd_{0,05}$=89,802 and $lsd_{0,01}$=136,047) show that in the foundation Tripofan Medium variant III differs very significantly individually from variants II and IV, which do not differ among themselves statistically. The number of bacteria isolated on the foundation Nutritious Agar with variants II and III statistically does not differ significantly, isolated with variants III and IV significantly differs and isolated with variants II and IV very significantly differs ($lsd_{0,05}$=136,386 and $lsd_{0,01}$=206,614).

Table 2. Main statistical indices of the number of Pseudomonas sp. bacteria strain isolated on the foundation Nutritious Agar in (000)

Varijants	Arithmetic mean	Variation interval	Standard deviation (SD)	Standard error (SE)	Variation coefficient
II	421,00	391-451	30,00	17,32	7,13
III	478,00	391-568	88,54	51,12	18,52
IV	664,33	582-718	72,39	41,79	10,90

Statistical significance of the difference in the number of bacteria isolated with the same variant on different foundations was tested with t-test for independent samples. Sampling t-values (t_{II}=3,558; t_{III}=2,469 and t_{IV}=6,288) show that the difference in the number of bacteria isolated with the third variant is not statistically significant, isolated with the second variant is statistically significant, while the number of bacteria isolated with the fourth variant changes very significantly with the change of foundation.

Biochemical characteristics of bacteria (isolates) show that the bacteria come from the strain *Pseudomonas sp*. (Table 3.). The strains from variant II (rhizosphere soil) and variant IV (root-sterilized) are biochemically absolutely identical. Some strains from

variant III (root-unsterilized) show certain particularity in biochemical respect. To be more exact, these strains of bacteria did not use citrates (negative citrates). According to API-20 they belong to *Pseudomona sp*.(8%) which exhibit this kind of reaction.

Picture 1.

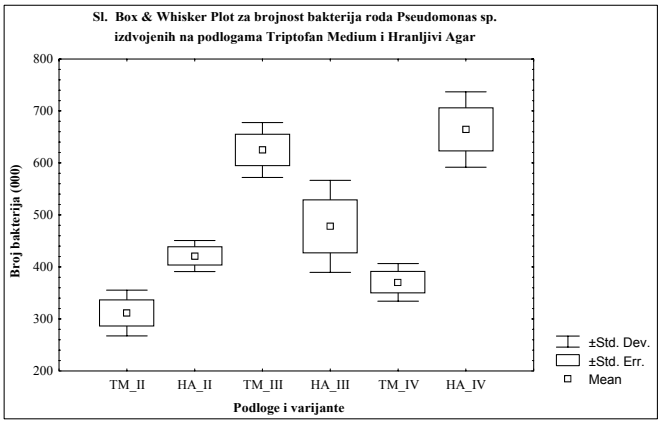

Table 3. Biochemical characteristics of Pseudomonas sp.

Variant	Isolate (strain)	glucose	gasa from glucose	lactosis	H_2S	manitola	mobility	indole	urea	citrates	oxidasis
II	1	–	–	–	–	–	+	–	–	+	–
	2	–	–	–	–	–	+	–	–	+	–
	3	–	–	–	–	–	+	–	–	+	–
	4	–	–	–	–	–	+	–	–	+	–
	5	–	–	–	–	–	+	–	–	+	–
III	**6**	–	–	–	–	–	+	–	–	–	–
	7	–	–	–	–	–	+	–	–	–	–
	8	–	–	–	–	–	+	–	–	–	–
	9	–	–	–	–	–	+	–	–	+	–
	10	–	–	–	–	–	+	–	–	+	–
IV	11	–	–	–	–	–	+	–	–	+	–
	12	–	–	–	–	–	+	–	–	+	–
	13	–	–	–	–	–	+	–	–	+	–
	14	–	–	–	–	–	+	–	–	+	–
	15	–	–	–	–	–	+	–	–	+	–

The bacteria which were isolated from root (variant IV) were probably located in intracellular spaces and in large numbers. These bacteria are especially interestinf, because they came in direct (physical) connection with the root. That is why we can consider them to be specific for this kind of vegetable (tomato).

CONCLUSION

- The number of *Pseudomonas sp.*is the highest in variant III on Triptofan medium and in variant IV on Nutrient agar. The smallist number of *Pseudomonas sp.* is in variant II on Triptofan medium and Nutrient agar.
- The number of isolated bacterias in different variants are statistical very different on Triptofan medium and statistical differentd on Nutrient agar.
- The isolated strains of bacteria show same biochemical properties. Strains 6,7 and 8 which are isolation from variant III did not use citrate (negativ citrate)
- The isolated strains are morphological same. They are Gram-negative, asporogeneous, rods.

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