Katarzyna GRATA[1]

EFFECT OF UREA PHOSPHATE ON THE *Bacillus* sp. POPULATION IN SOIL AND ANTIFUNGAL ACTIVITY OF SELECTED BACILLUS SPECIES ON *Fusarium* sp.

WPŁYW FOSFORANU MOCZNIKA NA LICZEBNOŚĆ BAKTERII Z RODZAJU *Bacillus* sp. W GLEBIE I AKTYWNOŚĆ PRZECIWGRZYBOWĄ WYBRANYCH SZCZEPÓW NA *Fusarium* sp.

Summary: The main objective of the examination was to assess the influence urea phosphate (UP) on the number of *Bacillus* sp. and antifungal activity both *Bacillus subtilis* and *Bacillus amyloliquefaciens* on *Fusarium* sp. Studies revealed positive influence of objects with urea phosphate on the number of *Bacillus* sp. The number of those microorganisms was higher than in the nonmanured and manured with FYM assayed throughout the experiment period. The spore germination of all *Fusarium* species were strongly inhibited but strain *Fusarium tricinctum* showed the highest sensitivity to metabolites of *Bacillus subtilis* I 7. *Bacillus amyloliqefaciens* III 14 demonstrate the highest activity against mycelium growth of all tested moulds.

Keywords: urea phosphate, *Bacillus* sp., antifungal activity, *Fusarium* sp.

Farmyard manure (FYM) and other materials of organic origin are applied to the soil in order to increase the levels of plant nutrients and improve the physical and chemical soil properties that directly affect soil fertility. Nevertheless, they can contain potentially pathogenic microorganisms for plants and people. The urea phosphate thanks to which one can improve the sanitary conditions of the fertilized soil turned out to be one of the effective disinfecting agents. Previous studies have demonstrated the favourable action to ammonificators, nitrificators, denitrificators and *Azotobacter* sp. [1].

Bacillus sp. and relatives have an affect on the natural environment, for example on the plant growth and on the pathogen occurring in soil. These bacteria has been shown capable of increasing nutrient availability in the rhizosphere (*B. megaterium*), able to fix atmospheric nitrogen and produce plant growth regulators (auxin, cytokinins and/or giberelins). Besides, *Bacillus* sp. and relatives are well known to possess antagonistic activities against many soilborne fungal and bacterial plant diseases by production a wide range of metabolites [2-5].

Example, include bacitracins A-F by *B. licheniformis,* bacilliomycin D, subtilin and iturins by *B. subtilis* as well as zwittermicin by *B. cereus.* In addition, several unidentified antibacterial and/or antifungal compounds are also produced by *B. cereus, B. subtilis, Penibacillus polymyxa, B. circulans* and *B. coagulans* [2, 6-9]. These compounds have been implicated in biocontrol of fungal pathogens such as *Rhizoctonia solani, F. oxysporum, Botritis cinerea, Sclerotina sclerotinum* [5]. Apart from antibiotics, *Bacillus* sp. may antagonize pathogens include production of fungal cell wall lytic enzymes (chitinase), they was produced by *B. cereus* and was effective in suppressing *Rhizoctonia solani* [10]. The aim of this study was to estimate the influence of urea phosphate on *Bacillus* sp. population

[1] Biotechnology and Molecular Biology Department, University of Opole, ul. kard. B. Kominka 4, 45-035 Opole, tel. 077 401 60 56, email: kgrata@uni.opole.pl

in the soil and the antifungal properties of the *Bacillus subtilis* and *Bacillus amyloliquefaciens* against 4 species of *Fusarium.*

Materials and methods

The studied material consisted of samples of brown soil, pH_{KCl} 5.5, sampled from the layer of soil from 0 to 25 cm in the autumn period. The pot experiment design included four treatments:

1) control soil, without fertilization (S),
2) soil + farmyard manure (FYM),
3) soil + urea phosphate (S+UP),
4) soil + urea phosphate + farmyard manure (S+UP+FYM).

Soil was watered to 60% of total water capacity and incubated at the temperature of 20ºC, with moisture content being kept constant over the entire experimental period. Analyses were carried out in three replications, in: day 7 - I term, day 30 - II term and day 90 of the experiment - III term and determined the number of *Bacillus* sp. on DDG medium. The number of *Bacillus* sp. was assayed with the incubation-plate method. Bacterial strains were selected among a collection of 45 *Bacillus* sp. isolates from samples of soil fertilized with urea phosphate whereas fungal strains (*Fusarium* sp.) from soil fertilized with farmyard manure. The *Bacillus* were identified as strains of *Bacillus subtilis* - No B I 7 and B II 11 and *Bacillus amyloliquefaciens* - B III 14. The antagonistic activity of the *Bacillus* strains was evaluate on the basis of fungal spore germination and rate index of fungal growth. Analysis of the effect of these bacteria on fungal spore germination was performed with the modyfying slide germination method and was presented as a rate index of conidia germination [11]. Determination of influence of *Bacillus subtilis* and *Bacillus amyloliquefaciens* on mycelium growth was estimated as the rate index of fungal growth [12].

Results and discussion

The influence of urea phosphate on *Bacillus* growth and antifungal activity of selected *Bacillus* strains was studied.

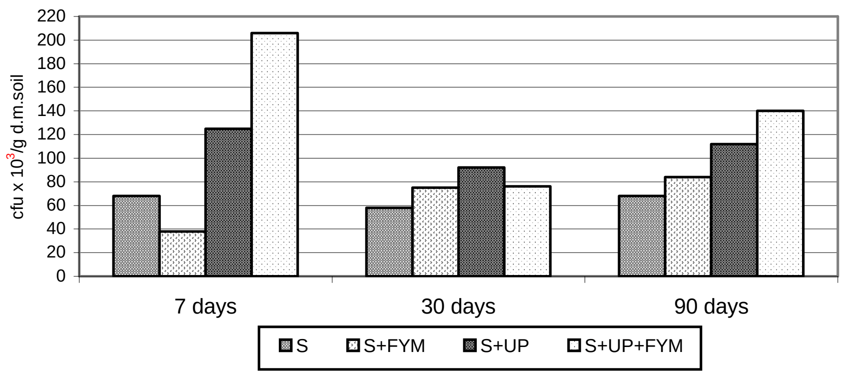

Fig. 1. Number of *Bacillus* sp. in 1 g d.m. of soil depending on fertilization

Changes in the numbers of *Bacillus* sp. in soil are presented in Figure 1. In the presence of urea phosphate (in S+UP and S+UP+FYM objects) higher count of *Bacillus* sp. was observed when compared with objects containing no urea phosphate or farmyard manure. The highest increase of the number of those bacteria after 7 days ($206\cdot10^3$ cfu/g) and 90 days ($140\cdot10^3$ cfu/g) was recorded in fertilized soil (S+UP+FYM). Similarly, the greatest growth of *Bacillus* sp. was observed in non-fertilized soil (S+UP) $125\cdot10^3$ cfu/g after 7 days and $112\cdot10^3$ cfu/g after 90 days.

Simultaneously, in the objects containing FYM alone, their number was higher after 30 and 90 days, than in the nonmanured soil.

In the present work, antifungal effect of *B. subtilis* and *B. amyloliquefaciens* was determined as the fungal spore germination and the rate index of fungal growth.

The degree of fungal spore germination was different, depending on the strains of *Bacillus sp.* and also age of the culture applied. The results are shown in Table 1. In this experiment 6, 12 and 24 h cultures of *Bacillus* sp. were used as an inhibition factor. The strongest inhibition of fungal spore germination was observed when *B. subtilis* I 7 was used as 12 or 24 h, *B. subtilis* II 11 as 6 h cultures against all studied fungal species. The spore germination of *Fusarium tricinctum* was strongly inhibited by both *B. subtilis* strains and *Fusarium solani* by *B. amyloliquefaciens* whereas the spore germination of *Fusarium oxysporum* and *Fusarium sporotrichoides* were weakly inhibited by all of studied *Bacillus* strains. The *B. subtilis* I 7 was more efficient than the others *Bacillus* strains.

Table 1

The index rate germination of *Fusarium* species [%]

Bacillus strain		***Fusarium solani***	***Fusarium tricinctum***	***Fusarium oxysporum***	***Fusarium sporotrichoides***
***Bacillus subtilis* I 7**	**control**	96.69	97.73	97.23	100.00
	6 h	94.11	78.50	94.49	80.00
	12 h	88.63	41.66	93.80	86.35
	24 h	76.92	92.22	84.42	87.50
***Bacillus subtilis* II 11**	**control**	96.69	97.73	97.23	100.00
	6 h	93.55	66.66	85.71	84.61
	12 h	95.00	94,61	100.00	93.38
	24 h	92.38	90,52	92.70	95.04
***Bacillus amyloliquefaciens* III 14**	**control**	96.69	97.73	97.23	100.00
	6 h	95.00	90.00	92.46	100.00
	12 h	78.99	86.54	97.17	100.00
	24 h	61.11	97.65	95.45	100.00

The antifungal activity of *Bacillus* sp. grown on 2 different media was evaluated towards *Fusarium* sp. as the rate index of fungal growth.

Table 2 shows the results obtained with PDA medium. Among the *Fusarium* species, *F. solani, F. oxysporum, F. sporotrichoides* were the most sensitive to metabolites produced by *B. amyloliquefaciens* III 14 (6 and 24 h old culture) whereas *F. tricinctum* by *B. subtilis* II 11 (6 h old culture) (Table 2).

Table 2

The influence of *Bacillus subtilis* and *Bacillus amyloliqefaciens* on *Fusarium* growth on PDA medium (diameter of the mycelium in mm)

Bacillus strain		***Fusarium solani***	***Fusarium tricinctum***	***Fusarium oxysporum***	***Fusarium sporotrichoides***
***Bacillus subtilis* I 7**	**control**	33.03	25.71	34.41	34.01
	6 h	32.00	27.68	32.08	31.32
	12 h	32.33	31.94	33.69	31.06
	24 h	27.16	28.69	35.16	29.54
***Bacillus subtilis* II 11**	**control**	33.03	25.71	34.41	34.01
	6 h	25.24	24.14	25.68	18.02
	12 h	25.09	24.26	23.68	19.47
	24 h	26.29	26.26	24.01	17.01
***Bacillus amyloliquefaciens* III 14**	**control**	33.03	25.71	34.41	34.01
	6 h	21.58	29.06	20.06	14.59
	12 h	22.34	25.51	20.30	16.06
	24 h	22.21	24.43	20.37	14.32

The results obtained with Czapek-Dox medium are reported in Table 3. Growth of *Fusarium solani* and *Fusarium sporotrichoides* was strongly inhibited, whereas growth of *Fusarium tricinctum* was poorly inhibited by all *Bacillus* strains.

It was found, that the inhibitory activities of *B. amyloliquefaciens* III 14 was significantly higher than all the other *Bacillus* strains tested.

Table 3

The influence of *Bacillus subtilis* and *Bacillus amyloliqefaciens* on *Fusarium* growth on Czapek-Dox medium (diameter of the mycelium in mm)

Bacillus strain		***Fusarium solani***	***Fusarium tricinctum***	***Fusarium oxysporum***	***Fusarium sporotrichoides***
***Bacillus subtilis* I 7**	**control**	36.62	35.56	36.76	38.31
	6 h	27.98	36.47	31.94	30.31
	12 h	29.41	36.95	27.72	30.54
	24 h	29.60	36.78	36.76	29.40
***Bacillus subtilis* II 11**	**control**	36.62	35.56	36.76	38.31
	6 h	11.33	34.07	26.94	28.03
	12 h	11.79	34.43	26.09	27.28
	24 h	25.48	34.82	30.06	26.00
***Bacillus amyloliquefaciens* III 14**	**control**	36.62	35.56	36.76	38.31
	6 h	17.54	35.65	29.16	27.46
	12 h	11.55	17.99	28.71	24.58
	24 h	25.30	32.13	29.60	22.53

Conclusions

1. Urea phosphate applied as the disinfectant of farmyard manure have the beneficial effect on the increase of the population of *Bacillus* sp. in soil.
2. The inhibitory properties of *Bacillus* species depend on: age of the culture applied and /or strains of *Bacillus.*
3. *Bacillus subtilis* (strains I 7 and II 11) had the highest inhibitory effect for spore germination *Fusarium tricinctum* whereas *Bacillus amyloliquefaciens* for *Fusarium solani.*

4. Among the *Fusarium* species, *Fusarium solani* and *Fusarium sporotrichoides* shows higher sensibility of mycelium to all tested *Bacillus* sp., whereas *Fusarium tricinctum* was a very little inhibited or stimulated.

References

[1] Grata K. and Krzyśko-Łupicka T.: Rocz. Glebozn., 2005, **LVI**(3/4), 13-20.
[2] Clanway C.P.: *Plant growth Promotion by Bacillus and Relatives*. [In:] R. Berkeley, M. Heyndriclex, N. Logen, P. De Vos: Applications an Systematic of Bacillus and Relatives. Blackwell Publishing 2002, pp. 219-236.
[3] Földes T., Bánhegyi I., Herpai Z., Varga L. and Szigeti J.: J. Appl. Microbiol., 2000, **89**, 840-846.
[4] Knox O.G.G., Killham K. and Leifert C.: Appl. Soil. Ecol., 2000, **15**, 27-231.
[5] Toure Y., Ongena M., Jacques P., Guiro A. and Thonart P.: J. Appl. Microbiol., 2004, **96**, 1151-1160.
[6] Czaczyk K., Trojanowska K. and Mueller A.: Acta Microbiol. Pol., 2002, **51**, 275-283.
[7] Czaczyk K., Bojanowska K. and Stachowiak B.: Polish J. Environ. Stud., 2002, **11**(5), 593-597.
[8] Kerr J.R.: Microb. Ecol. Health and Disease, 1999, **11**, 129-142.
[9] Myone A.L., Shelby R., Cleveland T.E. and Tuzun S.: J. Appl. Microbiol., 2001, **90**, 622-629.
[10] Wang S-L., Shih I-L., Wang C-H., Tseng K-C., Chang W-T., Twu Y-K., Ro J-J. and Wang C-L.: Enzyme Microbiol. Technol., 2002, **31**, 321-328.
[11] Burgieł Z. and Kończyk D.: Acta Agrar. Silvestr., Ser. Agraria, 1989, **XXVIII**, 25-31.
[12] Burgieł Z.: Acta Agrar. Silvestr., Ser. Agraria, 1984, **XXIII**, 187-195.

WPŁYW FOSFORANU MOCZNIKA NA LICZEBNOŚĆ BAKTERII Z RODZAJU *Bacillus* sp. W GLEBIE I AKTYWNOŚĆ PRZECIWGRZYBOWĄ WYBRANYCH SZCZEPÓW NA *Fusarium* sp.

Streszczenie: Badano wpływ fosforanu mocznika (FM) na liczebność bakterii z rodzaju *Bacillus* i ocenę aktywności fungistatycznej zarówno *Bacillus subtilis*, jak również *Bacillus amyloliquefaciens* na *Fusarium* sp. Przeprowadzone badania wykazały korzystne oddziaływanie obiektów z udziałem fosforanu mocznika na liczebność bakterii z rodzaju *Bacillus* sp. W ciągu całego okresu badań liczebność ich była większa niż w glebie nienawożonej i nawożonej obornikiem. Zdolność kiełkowania zarodników grzybów z rodzaju *Fusarium* była hamowana przez *Bacillus* sp., lecz największą wrażliwość na *Bacillus subtilis* I 7 wykazał *Fusarium tricinctum*. Natomiast największą aktywność w stosunku do grzybni wszystkich testowanych grzybów wykazał *Bacillus amyloliquefaciens* III 14.

Słowa kluczowe: fosforan mocznika, *Bacillus* sp., aktywność przeciwgrzybowa, *Fusarium* sp.