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POSSIBILITY OF USING *Bacillus* AND *Trichoderma* STRAINS FOR DECOMPOSITION OF CROP RESIDUES

ABSTRACT: The objective of this study was to investigate the possibility of using microbial strains as residue decomposers and to determine the effect of these strains on chemical and microbial properties in the residue-amended soil. Greenhouse experiment consisted of eight *Bacillus* treatments, three *Trichoderma* treatments, and their combination, all applied to non-sterile chernozem soil amended with wheat straw. Incorporation of wheat straw improved soil chemical and microbial properties, while the extent of residue decomposition under microbial strains was intensified. Microbial treatments significantly affected the soil pH, the content of carbonate, total carbon, soil organic carbon, humus, and available phosphorus and potassium. Bacterial and fungal treatments also significantly influenced the total microbial number, ammonifiers, N_2-fixers, fungi, actinomycetes, oligotrophs, copiotrophs, and cellulolytic microorganisms. The effect of microbial treatments varied depending on the applied strains and examined properties, with *Bacillus* strains being more promising residue decomposers compared to *Trichoderma* strains. The most effective microbial strains could be used as potential decomposers of crop residues.

KEYWORDS: *Bacillus*, soil microorganisms, soil organic matter, *Trichoderma*, wheat straw

INTRODUCTION

A sustainable agroecosystem relies upon an adequate amount of soil organic matter (SOM). Organic matter plays an important and multiple roles in soil, affecting physical, chemical, and biological soil properties, such as soil structure, cation exchange capacity, soil pH, and nutrient and energy supply for microbial biomass and higher plants (Walsh and McDonnell, 2012). The amount of organic matter in soil is largely influenced by cropping (Liu et al., 2005). Management practices such as repetitive tillage, removal or burning of crop residues, and

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inadequate fertilization result in a decrease of SOM content (Ghimire et al., 2017). Conversely, increasing or preserving the SOM content requires a sustained effort that includes reduced or no-tillage, crop residue amendment, integration of organic and chemical fertilizers, and crop rotations (Shrestha et al., 2013).

Incorporation of crop residues or other forms of organic material may reduce the application of chemical fertilizers while maintaining SOM levels and improving fertility and productivity of agricultural soils (Singh and Rengel, 2007). A great proportion of nutrient input during cultivation i.e. 30–35% of applied nitrogen (N) and phosphorus (P), and 70–80% of applied potassium (K), remains in crop residues (FAO, 2006). For instance, incorporation of wheat straw saves 50% of the recommended fertilizer quantity, followed by increased productivity of crops (Rajput, 1995). Additionally, crop residues are the main contributor to soil organic carbon (SOC) pool, which is a key index for soil fertility and a major carbon (C) reservoir in an agroecosystem (Manlay et al., 2007). Soil organic matter dynamics depends on the balance between C inputs via return of crop residues or organic amendments and C outputs primarily through SOC decomposition (Wang et al., 2015).

Crop residues are composed of lignin, cellulose, hemicellulose, and nutrients. The decomposition of crop residues is primarily determined by soil microorganisms (Schneider et al., 2012). Burning or removal of residues can decrease microbial biomass and microbial activity compared with soils where crop residues are returned to the soil (Chowdhury et al., 2015). Since soil microbial communities are the main regulators of nutrient cycling and soil carbon processes, differences in their composition have the potential to influence the provisioning of crop nutrients and the retention of C in residue-amended soils (Bending et al., 2002). The intensification of microbial and chemical processes during decomposition can be achieved using microbial strains as potential residue decomposers (Miki et al., 2010).

Species of *Bacillus* and *Trichoderma* are widely distributed in soil and well known for their beneficial effects on crop productivity (Bhattacharyya et al., 2016). They are among the most investigated biocontrol and plant growth-promoting agents that contribute to the suppression of plant pathogens and enhancement of plant growth (Abhilash et al., 2016). However, little is known about the impact of these microbial strains on residue decomposition and their relation to the soil microbiome during this process. Therefore, the objective of this study was to investigate the possibility of using *Bacillus* and *Trichoderma* strains as residue decomposers and to determine the effect of microbial treatments on chemical and microbial properties in the residue-amended soil.

MATERIALS AND METHODS

Microbial strains

Bacillus strains used in this study were obtained from the collection of the Department of Microbiological Preparations, Institute of Field and Vegetable

Crops, Novi Sad, Serbia. The strains were originally isolated from the soil samples, which included the rhizosphere of plants, agricultural and non-agricultural soils from different locations in the Province of Vojvodina. The bacteria were cultured for 48 h in nutrient broth (NB) at 30 °C to obtain the 10^9 cells ml^{-1} inoculum density. *Trichoderma* strains originated from the soil samples collected at Rimski šančevi experimental field of the Institute of Field and Vegetable Crops. The fungi were grown on potato dextrose agar (PDA) for seven days at 27 °C. After incubation, a sample of each fungus was taken and suspended in sterile distilled water to prepare the 10^9 spores ml^{-1} inoculum density.

Greenhouse experiment

The experiment was conducted in plastic boxes (36 × 28 × 15 cm) in a greenhouse under non-sterile, ambient temperature conditions. Each box contained 4 kg of non-sterile chernozem soil, 50 g of wheat straw, and 750 ml of microbial inoculum. Experiment included eight bacterial treatments: *Bacillus safensis* (BS), *Lysinibacillus fusiformis* (LF), *Bacillus megaterium* (BM), *Bacillus thuringiensis* (BT), *Lysinibacillus sphaericus* (LS), *Bacillus pumilus* (BP), *Bacillus cereus* (BC), and *Bacillus* mixture (Bmix); three fungal treatments: *Trichoderma harzianum* (TH), *Trichoderma asperellum* (TA), and *Trichoderma* mixture (Tmix); and a combination of bacterial and fungal mixture (BTmix). Mixtures consisted of bacterial and/or fungal strains mixed in an equal ratio. The effect of applied microbial treatments was compared to soil and soil with wheat straw, both with 750 ml of water (control). There were three replications of each treatment. The experiment was conducted for six months and watered weekly to maintain optimal conditions for microbial activity.

Soil chemical analysis

Soil samples for chemical analysis were collected at the end of the experiment (after 180 days). The samples were dried at the room temperature, milled and sieved to a particle size of < 2 mm. Chemical properties of examined soil were determined at the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops, using standard methods. The pH value in 1:5 (v/v) of soil suspension in 1 mol l^{-1} KCl was determined potentiometrically. Carbonate content ($CaCO_3$) (%) was measured with a Scheibler calcimeter. Contents of total nitrogen (N) (%), total carbon (C) (%), and soil organic carbon (SOC) (g/kg) were obtained via CHNS elemental analysis (Vario EL III, Elementar). The humus content (%) was assessed by oxidation of organic matter by the method of Tyurin. Available K_2O and P_2O_5 (mg/100 g) were determined by extraction with ammonium lactate according to Egner-Riehm. Potassium content (K) was determined using the flame photometer (Evans Electroselenium Ltd.). Phosphorus (P) content was analyzed using the blue method in a spectrophotometer (Agilent Cary 60, Agilent Technologies). All chemical analyses were performed in four replicates.

Soil microbial analysis

Soil samples for microbial analysis were collected four times during the experiment (after 45, 90, 130, and 180 days). The microbial number was analyzed at the Department of Microbiological Preparations of the Institute of Field and Vegetable Crops, using indirect dilution method followed by plating of soil suspension on selective nutritive media. The total number of microorganisms (TNM) was determined on an agarized soil extract, ammonifiers (AMN) on a meat-peptone agar (MPA), and free N_2-fixers (NFB) on a nitrogen-free agar. The number of fungi (FNG) was determined on Czapek-Dox agar, actinomycetes (ACT) on synthetic agar, oligotrophic (OB) and copiotrophic (CB) bacteria on (C)- poor and (C)- rich medium, and cellulolytic microorganisms (CEL) on Waksman-Carey medium. The incubation temperature was 28 °C, while the incubation time depended on the tested group of microorganisms (Jarak and Đurić, 2006). The average number of colony-forming units (CFU) was calculated per 1.0 g of absolute dry soil. All microbial analyses were performed in four replicates.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Means were compared using Tukey's honest significant difference (HSD) test at the $P < 0.05$ level. All analyses were performed in STATISTICA 12.6 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Microbial strains used in this study were selected according to a previously performed screening of bacteria and fungi for potential cellulolytic activity using enzymatic and dry fermentation assays (data not shown). Chemical analysis showed that experimental soil was slightly alkaline and slightly humic, with a high content of easily accessible phosphorus and optimal supply of easily accessible potassium. Significant differences between soil and soil amended with wheat straw were observed for pH, SOC, and K (Table 1). Applied microbial treatments significantly affected all examined soil chemical properties except total N (Table 1). All *Bacillus* treatments increased the pH values, while *Trichoderma* treatments had a lower pH compared to control. A significant increase in pH was recorded with *Bacillus safensis*, *Bacillus cereus,* and *Bacillus* mixture. *Trichoderma harzianum* significantly increased the content of $CaCO_3$, while other treatments had a negligible impact on the carbonates. Similarly, significant differences in N content between experimental treatments were not recorded. Bacterial treatments positively affected the total C and SOC, while a significant increase was observed in all *Bacillus* treatments, except *Bacillus cereus*. Conversely, fungal treatments slightly decreased the content of total C and

significantly decreased the content of SOC. All *Bacillus* individual treatments, as well as *Trichoderma harzianum* and *Bacillus-Trichoderma* mixture treatments significantly affected the humus content. A significant increase in P and K content was recorded with *Bacillus* individual treatments and both *Bacillus* mixtures. According to Ogbodo [2011], organic matter from residue improved the soil pH status by increasing the soil buffer capacity. An increase in soil pH followed by an increase in nutrient content in residue–amended soil is frequently reported [Butterly et al., 2013]. The organic matter acts as a storage from which basic cations are released into the soil solution, while the improved soil nutrient status leads to better soil quality and crop productivity [Manlay et al., 2007].

Table 1. Chemical properties of residue-amended soil depending on examined microbial treatments

Treatment	pH	$CaCO_3$ (%)	Total N (%)	Total C (%)	SOC (g kg^{-1})	Humus (%)	P (mg)*	K (mg)*
Soil	7.35 f	0.93 bc	0.184 a	1.95 d	17.03 h	2.48 h	26.1 d	22.7 c
Control	7.56 cde	1.17 bc	0.189 a	2.06 cd	18.69 f	2.55 gh	25.2 d	30.5 b
BS	7.70 ab	1.17 bc	0.208 a	2.24 ab	19.74 b	2.80 ab	39.3 a	38.2 a
LF	7.68 abc	0.93 bc	0.203 a	2.22 ab	19.10 c	2.73 bcd	35.0 bc	39.1 a
BM	7.64 bcd	1.17 bc	0.200 a	2.27 a	20.31 a	2.69 cdef	34.0 bc	38.6 a
BT	7.65 abcd	0.93 bc	0.205 a	2.23 ab	19.59 b	2.76 abc	32.3 c	40.0 a
LS	7.68 abc	1.17 bc	0.205 a	2.18 ab	19.08 cd	2.76 abc	34.3 bc	40.0 a
BP	7.68 abc	1.17 bc	0.210 a	2.18 ab	18.89 e	2.83 a	32.5 c	38.6 a
BC	7.71 ab	0.93 bc	0.207 a	2.15 bc	18.68 f	2.78 abc	32.6 c	38.6 a
BMix	7.77 a	1.35 b	0.195 a	2.22 ab	18.93 de	2.63 efg	32.7 c	40.9 a
TH	7.51 e	4.20 a	0.197 a	1.96 d	18.31 g	2.65 def	24.0 d	29.1 b
TA	7.54 de	1.35 b	0.193 a	1.99 d	18.38 g	2.60 fg	26.6 d	30.5 b
TMix	7.48 e	1.17 bc	0.194 a	1.99 d	18.36 g	2.61 fg	26.0 d	30.0 b
BTmix	7.68 abc	0.75 c	0.201 a	2.17 abd	18.54 f	2.71 bcde	35.8 b	38.2 a
P	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000

Values are the means of 4 replicates. Values in a column with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. *In a 100 g sample of soil. BS – *Bacillus safensis*; LF – *Lysinibacillus fusiformis*; BM – *Bacillus megaterium*; BT – *Bacillus thuringiensis*; LS – *Lysinibacillus sphaericus*; BP – *Bacillus pumilus*; BC – *Bacillus cereus*; Bmix – *Bacillus* mixture; TH – *Trichoderma harzianum*; TA – *Trichoderma asperellum*; Tmix – *Trichoderma* mixture; BTmix – *Bacillus* and *Trichoderma* mixture.

Different cropping systems can have a positive or negative effect on microbial number and activity, which directly reflect the fertility of the soil. Our research revealed that wheat straw amendment increased the presence of all microbial communities, and led to a significant change in the total

microbial number, number of ammonifiers, N_2-fixers, fungi, and copiotrophs compared to soil without crop residues. Microbial treatments significantly affected the number of all examined microbial groups (Table 2). All microbial treatments, except *Trichoderma harzianum*, increased the total microbial number. A significant increase in the total microbial number was achieved in all treatments apart from *Bacillus safensis*. The number of ammonifiers and N_2-fixers was increased in applied treatments, while the significant effect was observed for all treatments but *Trichoderma harzianum*. The number of fungi was significantly increased with *Trichoderma harzianum*, while an increase in the number of this microbial group was also recorded with *Bacillus safensis*, *Bacillus cereus*, *Trihoderma asperellum,* and both *Trichoderma* mixtures. Interestingly, treatments with *Lysinibacillus fusiformis*, *Bacillus megaterium*, and *Bacillus thuringiensis* led to a significant decrease in the number of fungi. The number of actinomycetes was significantly higher in *Lysinibacillus fusiformis, Bacillus cereus*, and *Trichoderma* mixture treatments, while their population was not considerably altered in other experimental treatments. All microbial treatments, except *Trihoderma harzianum*, positively affected the number of oligotrophs, while a significant increase was obtained with *Bacillus safensis*, *Bacillus cereus*, and *Trichoderma* mixture. The number of copiotrophs was higher in all microbial treatments when compared to control, while the significant effect on this microbial group was recorded in *Bacillus* individual treatments, *Bacillus* mixture, and *Trichoderma asperellum* treatments. Cellulolytic microorganisms were significantly increased with *Lysinibacillus sphaericus, Bacillus mixture, Trichoderma asperellum*, and *Trichoderma* mixture, while a positive effect was obtained in most other treatments.

Examined microbial parameters in this study are important indicators of nutrient cycling and carbon processes, as well as the soil perturbations during residue decomposition. Ammonifiers degrade organic nitrogen compounds, while nitrogen-fixing bacteria reduce atmospheric nitrogen (Isobe et al., 2014). Actinomycetes and fungi are effective at decomposing complex organic compounds including lignin and cellulose (Bai et al., 2016). Copiotrophs and oligotrophs indicate the availability of carbon source, while cellulolytic microorganisms are the main decomposers of plant biomass (Ho et al., 2017). In this study, higher microbial abundance was accompanied by higher enzyme activities (data not shown). The higher enzyme activities may coincide with greater capacity to produce enzymes by the larger microbial biomass or might be partially attributed to higher substrate quantity and complexity (Yang et al., 2011). Previous studies reported the importance of crop residue returns for improving microbial community structure and maintaining soil fertility (Pascault et al., 2010; Arcand et al., 2016), while this research emphasized its significance through the application of adequate microbial strains.

Table 2. Microbial properties of residue-amended soil depending on examined treatments

Treatment	TNM (CFU×10^6)	AMN (CFU×10^6)	NFB (CFU×10^5)	FNG (CFU×10^3)	ACT (CFU×10^3)	OB (CFU×10^6)	CB (CFU×10^6)	CEL (CFU×10^5)
	g^{-1} soil							
Soil	107 h	67 i	60 f	17 efg	0 d	191 g	251 g	4 d
Control	344 f	166 h	197 e	24 bcde	2 cd	310 defg	529 f	21 cd
BS	399 ef	394 def	269 d	28 abcd	2 cd	461 abc	649 de	27 cd
LF	445 de	397 def	361 bc	12 gh	11 a	501 ab	677 de	41 bc
BM	446 de	420 cde	389 b	15 fgh	3 cd	384 bcde	676 de	40 bc
BT	509 bc	404 def	372 bc	9 h	3 cd	337 cdef	655 de	39 bc
LS	510 bc	609 a	603 a	21 def	2 cd	371 cdef	841 ab	58 ab
BP	597 a	493 b	397 b	18 efg	0 d	349 cdef	928 a	20 cd
BC	562 ab	370 ef	329 c	30 abc	6 b	266 efg	818 bc	26 cd
BMix	564 ab	477 bc	358 bc	22 cdef	1 cd	428 abcd	734 cd	54 ab
TH	245 g	216 gh	205 e	36 a	2 cd	249 fg	589 ef	19 cd
TA	489 cd	244 g	407 b	31 ab	1 cd	352 cdef	700 d	60 ab
TMix	519 bc	347 f	380 bc	28 abcd	4 c	541 a	538 f	79 a
BTmix	491 cd	457 bcd	259 d	25 bcde	1 cd	359 cdef	589 ef	20 cd
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Values are the means of 4 replicates. Values in a column with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. TNM: total microbial number, AMN: ammonifiers, NFB: N_2-fixers, FNG: fungi, ACT: actinomycetes, OB: oligotrophs, CB: copiotrophs, CEL: cellulolytic microorganisms. BS – *Bacillus safensis*; LF – *Lysinibacillus fusiformis*; BM – *Bacillus megaterium*; BT – *Bacillus thuringiensis*; LS – *Lysinibacillus sphaericus*; BP – *Bacillus pumilus*; BC – *Bacillus cereus*; Bmix – *Bacillus* mixture; TH – *Trichoderma harzianum*; TA – *Trichoderma asperellum*; Tmix – *Trichoderma* mixture; BTmix – *Bacillus* and *Trichoderma* mixture.

CONCLUSIONS

Our study confirmed the importance of residue incorporation and microbial decomposition in agricultural soil. Incorporation of wheat straw had a positive effect on examined soil properties, while microbial treatments improved chemical properties and increased microbial number. Overall, *Bacillus* treatments had a better effect on examined soil properties compared to *Trichoderma* treatments. Individual *Bacillus* strains mostly had an advantage over their combined application, while *Trichoderma* strains had the best effect in the mixtures. The most effective microbial strains could be used for decomposition of wheat straw in soils with similar properties. A further selection of microbial strains through greenhouse and field trials will be necessary to establish their efficiency as individual and combined decomposers of various crop residues in different soil environments.

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МОГУЋНОСТ КОРИШЋЕЊА *Bacillus* И *Trichoderma* СОЈЕВА ЗА РАЗЛАГАЊЕ ЖЕТВЕНИХ ОСТАТАКА

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РЕЗИМЕ: Циљ рада био је да се утврди могућност коришћења микробиолошких сојева у разлагању жетвених остатака, као и ефекат ових сојева на хемијска и микробиолошка својства у земљишту са додатком жетвених остатака. Оглед у стаклари обухватио је осам *Bacillus* третмана, три *Trichoderma* третмана и њихову смешу, који су примењени у нестерилном земљишту типа чернозем са пшеничном сламом. Уношење сламе побољшало је хемијска и микробиолошка својства земљишта, док је примена микробиолошких сојева интензивирала разградњу жетвених остатака. Микробиолошки третмани значајно су утицали на pH земљишта, садржај карбоната, укупног угљеника, органског угљеника, хумуса, приступачног фосфора и калијума. Третмани са бактеријама и гљивама такође су значајно утицали на укупан број микроорганизама, бројност амонификсатора, азотофиксатора, гљива, актиномицета, олиготрофа, копиотрофа и целулолитичких микроорганизама. Ефекат примењених третмана варирао је у зависности од примењеног соја и испитиваних својстава, при чему су сојеви *Bacillus*-а имали бољи ефекат у разградњи жетвених остатака у поређењу са *Trichoderma* сојевима. Најефикаснији микробиолошки сојеви могу се користити као потенцијални разлагачи жетвених остатака.

КЉУЧНЕ РЕЧИ: *Bacillus*, земљишни микроорганизми, органска материја земљишта, *Trichoderma*, пшенична слама