ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 107, No. 2 (2020), p. 105–112
DOI 10.13080/z-a.2020.107.014

Biological control potential of rhizosphere bacteria with ACC-deaminase activity against *Fusarium culmorum* in wheat

Gül IMRIZ[1], Fatih ÖZDEMIR[2], Mehmet Sait KARACA[2], Murat Nadi TAŞ[2], İlker TOPAL[2], Birol ERCAN[2]

[1]Dicle University, Faculty of Agriculture
Diyarbakır, Turkey
E-mail: gulimriz@hotmail.co.uk

[2]Bahri Dağdaş International Agricultural Research Institute
Konya, Turkey

Abstract

Fusarium culmorum (W.G. Smith) is one of the fungal soil-borne plant pathogens causing significant yield and quality losses in cereals. Chemical attempts are not only insufficient for controlling such pathogens, but also they bring hazardous effects on the environment and living organisms. Therefore, environment-friendly plant-beneficial microorganisms including bacteria would replace chemical control agents as promising and sustainable pest management. Numerous studies showed that some strains of plant growth-promoting rhizobacteria comprising the enzyme ACC (1-aminocyclopropane-1-carboxylate)-deaminase could promote the plant growth acting as a biological control agent by lowering the level of excessive ethylene in plants exposed to biotic and abiotic stresses. This study was aimed to evaluate the rhizobacterial isolates obtained from rhizosphere of cereal plants in Konya and Karaman provinces in Turkey for potency of enzyme ACC-deaminase activity and *in vitro*/*in vivo* suppression ability on *F. culmorum*. In total 31 out of 463 rhizobacterial isolates successfully suppressed *in vitro* growth of *F. culmorum* on potato dextrose agar following dual-culture technique. Afterwards, the successful isolates were examined for ACC-deaminase activity using ACC as the sole nitrogen (N) source. Two isolates coded as Gu2 and 127b with the highest enzyme ACC-deaminase activity were included in pot trials under controlled conditions for assessing *in vivo* pathogen suppression ability on wheat seedlings. The *in vivo* pathogen suppression efficiency of Gu2 and 127b isolates was determined as 81.25% and 37.50%, respectively. It was determined that both rhizobacterial isolates belonged to *Bacillus* spp. with high reliable score based on MALDI Biotyper System classification results.

Key words: combat, enzyme, pathogen, rhizobacteria, soil, wheat.

Introduction

The human population in the world is predicted to continue to increase. In the next 50 years, it is expected that the worldwide population growth will be around 10 billion and that of food demand to be between 59–98%. Consequently, food production will be insufficient to feed people around the world. Providing adequate food for the growing world population will not be easy; various strategies and approaches are needed to be generated. In order to produce more food, more extensive agricultural land will be required; the usage of chemicals including fertilisers and pesticides will increase; the demand for transgenic products as well as usage of microorganisms that promote plant growth will rise (Glick, 2014).

Plant growth-promoting rhizobacteria (PGPR) are a group of beneficial bacteria living in the rhizosphere, the phyllosphere or in the plant tissues as entophytes (Ahemad, Kibret, 2014; Miliute et al., 2015). The PGPR can promote plant growth by direct and indirect mechanisms or a combination of both (Siddikee et al., 2010; Imriz et al., 2014). Indirect mechanisms comprise the suppression of pathogens through the production of antibiotics and extracellular hydrolytic enzymes and the action of siderophores, inducing systemic resistance (ISR), exo-polysaccharides production. Direct mechanisms involve making the natural nutrition source ready to use for plants including fixation of atmospheric nitrogen, solubilization of phosphorus, potassium and iron, production of siderophores, producing phytohormones like auxins, cytokinins and gibberellins (Ram et al., 2013; Ahemad, Kibret, 2014) or by the activity of 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, an enzyme, which can hinder the "plant stress ethylene" that is typically raised by a number of environmental stresses such as

Please use the following format when citing the article:
Imriz G., Özdemir F., Karaca M. S., Taş M. N., Topal İ., Ercan B. 2020. Biological control potential of rhizosphere bacteria with ACC-deaminase activity against *Fusarium culmorum* in wheat. Zemdirbyste-Agriculture, 107 (2): 105–112. DOI 10.13080/z-a.2020.107.014

flooding, extreme temperature, the presence of organic and inorganic toxicants, phytopathogens, drought or high salt concentrations (Cheng et al., 2007; Glick, 2014; Gamalero, Glick, 2015).

Ethylene is a crucial plant signalling molecule with significant roles in many of plant functions such as seed germination, root growth, root nodulation, flowering and fructification. However, when the plants are exposed to biotic or abiotic stressors, the ethylene hormone is over-synthesised and turns out as "plant stress ethylene", which inhibits plant growth and development. Various fungi and bacteria including PGPR encode the enzyme ACC-deaminase, which can convert the ACC, the precursor molecule of ethylene in plants, to α-ketobutyrate and ammonia and lower the levels of "plant stress ethylene" caused by many stressors (Cheng et al., 2007; Singh et al., 2015).

Foot and root rot disease causal organism *Fusarium culmorum* (W.G. Smith) is a soil-borne fungal pathogen limiting winter cereal production in the world including Turkey (Chekali et al., 2013; Erginbaş Orakçı et al., 2018). *F. culmorum* causing grain yield and quality losses has a wide range of host plants, mainly cereals, including wheat, barley, oats, rye, corn, sorghum and various grasses. The pathogen can survive for long years at lower temperatures as hyphae in plant residues and as chlamydospores in the soil matrix. The disease symptoms on the wheat plant are defined as necrosis and dry rot of the crown base in brown colour and whitehead formation, which is most severe, in particular, through the seasons with a wet start and dry climates (Matny, 2015). The water deficit in plants followed by limited water supply significantly increases disease severity in wheat caused by *F. culmorum* (Liu, Liu, 2016; Streit et al., 2016).

Moreover, the predicted climatic changes are expected to affect the pathogen development and survival rates as well as the host sensitivity against the pathogen (Elad, Pertot, 2014). In Central Europe, the expected warmer and more humid conditions will be favourable for *Fusarium* species including *F. culmorum* (Madgwick et al., 2011; Parikka et al., 2012). It was projected that with climate change the *Fusarium* epidemics would be more severe, especially in southern England, by the 2050s (Madgwick et al., 2011). Hence, *F. culmorum* might be a potentially threat agent with increasing importance for cereal production in arid and semi-arid regions as well as in temperate climate countries.

The control of such soil-borne diseases would be possible by chemicals, e.g., seed applications. Although chemical protection with fungicides is highly efficient, they do not control for long and exert hazardous effects on the environment and living organisms (Wegulo et al., 2011). As an alternative to synthetic fungicides, biological control of pathogens including the use of ACC-deaminase producing plant growth-promoting bacteria has been an emphasis in the plant protection studies (Glick, 2014). Pre-treatment of plants with ACC-deaminase containing plant growth-promoting bacteria can provide significant protection to plants against some of the ethylene caused damage from pathogen infection as well as water stress (Husen et al., 2011; Amutharaj et al., 2012; Nascimento et al., 2013). However, the available biocontrol agents in some cases fail in the field. Some factors such as environmental and ecological factors, formulation type and application method can affect biocontrol agent efficacy in the field (Labuschagne et al., 2010). Perhaps, an isolate bearing the enzyme ACC-deaminase may provide protection against multiple stress factors that may be faced through agricultural production.

This study was aimed to evaluate the rhizobacterial isolates obtained from rhizosphere of cereal plants in Konya and Karaman provinces in Turkey for potency of enzyme ACC-deaminase activity and *in vitro*/*in vivo* suppression ability on *F. culmorum*. We succeeded in getting a bacterial isolate belonging to *Bacillus* genera from the wheat rhizospheric soil with high biocontrol potential for the soil-borne pathogen of wheat and comprising ACC-deaminase activity, which is known crucial enzyme having role in promoting plant. This isolate may be included in combating with multiple plant stress factors including biotic (e.g., pathogen attack) and abiotic (e.g., drought and soil salinity). Hence, the finding of this study is relatively important for developing novel biocontrol agents to control the pathogen highly affected by climate change.

Materials and methods

Soil samples and rhizobacterial isolates. In April–May of 2015–2017, soil samples from the rhizosphere of cereal plants (wheat and barley) were collected from all districts of Konya and Karaman provinces in Turkey, where agricultural activities, in particular cereal production, are the most intensive. The samples were carefully collected, labelled and transferred to the laboratory of Bahri Dağdaş International Research Institute, Turkey in polythene bags through cooling system. Bacterial isolation was done from the rhizospheric soil samples by a serial dilution technique (Kusek, Cinar, 2013) on nutrient agar (Merck, Germany) plates incubated at 25 ± 2°C for 72–96 hours. After incubation period, every single colony showing morphologically difference was purified on nutrient agar. Each of purified bacterial isolate was coded and maintained on nutrient agar slants at +4°C and 50% glycerol at −20°C.

Screening for in vitro antifungal activity. In vitro antimicrobial activity of all isolates (Table 1) against *Fusarium culmorum* (W.G. Smith) was screened by using the dual-culture technique on the potato dextrose agar (PDA) (Merck, Germany). The *F. culmorum* isolate from winter wheat (Central Anatolia region of Turkey) was obtained from Prof. Dr. Berna Tunali's culture collection. The isolate was used for both *in vitro* and *in vivo* tests. A 5 mm diameter mycelial disc of *F. culmorum* culture (7 days old) was placed on PDA, 2 cm away from the edge of plate. A loopful of 48 h old bacterial culture grown in agar medium was streaked on the opposite side of the plate with three replicates for each of the isolate. Only sterile distilled water was used instead of bacterial culture for the control plates. Plates were incubated at 25 ± 2°C for 10 days, when the control was fully covered by fungi. The inhibition zones were measured in cm, and inhibition efficiency percentage (%) of fungal growth was calculated by using the following formula:

$$(R - r) / R \times 100,$$

where R is radial growth of the pathogen in control plate, r – radial growth of the fungal colony interacting with antagonistic bacteria.

Screening assay for enzyme ACC (1-aminocyclopropane-1-carboxylate)-deaminase activity. To obtain ACC (Sigma®) stock solution, 3 mM ACC was filter sterilised with 0.2 μm membrane filter

and stored at −20°C until the assays. Before experiments ACC thawed. The screening for ACC-deaminase activity assay was performed as defined by Shahzad et al. (2010). The isolates that showed excellent inhibition on the hyphal growth of pathogen on PDA were also screened for the ACC-deaminase activity based on the ability to use enzyme ACC as a sole nitrogen (N) source. All of 31 rhizobacterial isolates were grown on two N sources: ACC and $(NH_4)_2SO_4$ and one mineral source ($MgSO_4$), to observe the growth rate of each isolate for ACC. The isolates were grown in 5 mL of tryptic soy broth (TSB-Difco™, USA) at 25 ± 2°C for 48 h by shaking (100× rpm).

Cultures were diluted 10 times in sterilised 0.1 M $MgSO_4$ solution. Primarily, 120 µL of minimal salt medium (MSM) (Sigma, Germany) was added to each well in 96-well plate, which was divided into three parts vertically (4 lanes each section). In the first 4 lanes, 15 µL 0.1 M $MgSO_4$ as negative control, and in second 4 lanes, 15 µL 0.1 M $(NH_4)_2SO_4$ as positive control, were added. The wells in the third part of lanes were filled with 15 µL of 3 mM ACC. For inoculation of each well, 15 µL bacterial culture (~10^8 cells mL^{-1}) was used.

Optical density (OD) was measured after 48 h at 600 nm by a microplate reader Sunrise™ (Tecan, Switzerland). The OD value of ACC and $(NH_4)_2SO_4$ wells were compared along with $MgSO_4$ wells to determine the ability of bacteria to utilize ACC for their growth. The rhizobacterial isolates were divided into three groups: isolates with higher (group H: OD600 > 0.75), medium (group M: OD600 > 0.75–0.50) and lower (group L: OD600 < 0.50) ACC utilization rate depending upon their OD values at 600 nm for ACC substrate as compared to $(NH_4)_2SO_4$ (Shahzad et al., 2010). For each isolate, two replicates were applied and the averages were considered for evaluation. The isolates with a higher ACC utilization rate had an OD value for ACC substrate wells that were close to the OD value for wells $(NH_4)_2SO_4$ in the first 48 hours of growth. Similarly, isolates with moderate ACC utilization had a low OD value for ACC wells compared to $(NH_4)_2SO_4$ in the first 48 hours. Isolates with lower ACC utilization rate possessed the lowest OD value for ACC wells (near to the OD value of wells for $MgSO_4$) at the same time.

In vivo experimental design and management. The rhizobacterial isolates that gave the highest ACC-deaminase activity were included in the *in vivo* pot trials to evaluate their antagonistic efficiencies on wheat seedlings. For this purpose, the seeds were inoculated with rhizobacterial isolates (~10^8 cells mL^{-1}) and sown in pots containing soil infested with *F. culmorum*. The experiment was carried out in a growth room adjusted to 24 ± 2°C and 80% relative humidity by using sterile pots, 5 cm in diameter and 25 cm in depth, containing sterile soil mix (1:1 mix of gardening peat-sand).

In the assay, durum wheat (*Triticum durum* Desf.) cultivar 'Kızıltan-91' was used as it is known as susceptible to *F. culmorum* (Arıcı, 2014). Wheat seeds were sterilized by dipping in 1% NaOCl for 5 min. The seeds were then rinsed twice with sterile distilled water (SDW) and dried. The seeds were inoculated with rhizobacterial isolates by dipping the seeds in the bacterial suspensions at a density of ~10^8 cells mL^{-1} and shaken for 60 min at 80× rpm and then allowed to dry. For control, the seeds were dipped only in SDW for the same period. The whole process was carried out under sterile conditions in this stage.

In order to prepare soil inoculum, Petri dishes containing PDA were inoculated with a disc of *F. culmorum* and incubated for 10 days till macroconidia sporulation. Ten days old pathogen culture was mashed with SDW by using a mortar and strained by a sieve. Conidia concentration of the obtained suspension was checked under the light microscope by a hematocytometer. The soil mix was infested with the suspension adjusted ~1×10^6 conidia g^{-1} of soil (Lemmens et al., 1993). The rhizobacterial-inoculated seeds were sown immediately and the pots covered by a polyethene for 48 hours to provide most favourable humidity conditions for max pathogen colonization. This experiment was conducted as positive control (pathogen contaminated soil + SDW applied wheat seed) and negative control (sterile soil + SDW applied wheat seed) pots, rhizobacterial application pots (pathogen contaminated soil + rhizobacterial contaminated wheat seed) with 8 replications (each contained two plants) according to the randomized plot design.

Fifteen days after sowing the seeds, plants were scored using the 0–3 scale (0 – no disease, 1 – very slight brown necrosis, 2 – slight / moderate brown necrosis and 3 – extensive brown-black necrosis) based on coloured lesion length (cm) on the plants, which is a typical disease symptom developed by the foot and root rot pathogen *F. culmorum* (Arıcı, 2014). The disease severity percentage was calculated based on scale values (Townsend, Heuberger, 1943). The efficiencies of the rhizobacterial applications were calculated by Abbott (1925) formula using the means of disease severity in applications and positive controls:

$$\text{Disease severity (\%)} = \frac{\Sigma(n \times V)}{Z \times N} \times 100,$$

where n is planted samples in a certain degree of disease on the scale, V – scale value, Z – the highest scale value, N – total number of plant samples.

$$\text{Isolate efficiency (\%)} = \frac{X - Y}{X} \times 100.$$

where X is average disease severity in the positive control (%), Y – average disease severity of the applied parcels (%).

Identification of rhizobacterial isolates. The isolates grouped in group H for ACC substrate utilization were identified by MALDI Biotyper System (Bruker Daltonics Inc., USA) through service purchase from Faculty of Agriculture, University of Mustafa Kemal, Turkey.

Statistical analysis. Inhibition zone size, inhibition efficiency data and the *in vivo* efficiencies of the isolates were statistically analysed by one-way analysis of variance (ANOVA) using software package *SPSS* (IBM Inc., USA). The means were separated by the Tukey HSD test ($p < 0.05$).

Results and discussion

Isolates and in vitro antifungal activity. In total 463 rhizobacterial isolates were obtained from 215 soil samples collected from the districts of Konya and Karaman provinces, Turkey. Thirty-one out of 463 isolates exhibited antifungal properties on *F. culmorum* growth on PDA with inhibition zones (cm) ranging from 1.10 to 6.25. The codes, locations, host plants of isolates, *in vitro* assay data regarding means of inhibition zones

and efficiency percentages of isolates are summarized in Table 1. Ten isolates (coded as 219b, 233d, 213f, 127b, 95b, 123b, 127a, Gu2, 130a and 95e) showed higher success with efficiencies above 40% to suppress *F. culmorum* growth *in vitro*. There were statistically significant differences among the inhibition zone sizes ($F = 177.332$, df = 31, $P = 0.001$) and inhibition efficiencies ($F = 230.495$, df = 31, $P = 0.001$) of the isolates.

Table 1. Antifungal properties of the isolates on *Fusarium culmorum*

Isolate code	Location / district	Host plant of isolate	Means of inhibition zones cm	Inhibition efficiency %
219b	**Selçuklu**	**wheat**	**6.25** ± 0.144 a	**69.4*** ± **1.60 a**
233d	**Selçuklu**	**wheat**	**6.10** ± 0.057 a	**67.7*** ± **0.64 a**
213f	**Kadınhanı**	**barley**	**5.90** ± 0.100 a	**65.5*** ± **1.11 a**
127b	**Ilgın**	**wheat**	**4.75** ± 0.144 b	**52.7*** ± **1.60 b**
95b	**Güneysınır**	**wheat**	**4.75** ± 0.144 b	**52.7*** ± **1.60 b**
123b	**Ilgın**	**wheat**	**4.50** ± 0.000 b	**50.0*** ± **0.00 bc**
127a	**Ilgın**	**wheat**	**4.25** ± 0.144 bc	**47.2*** ± **1.60 bcd**
Gu2	Çumra	**wheat**	**4.10** ± 0.100 bcd	**45.5*** ± **1.11 cde**
130a	**Akşehir**	**wheat**	**4.10** ± 0.057 bcd	**45.5*** ± **0.64 cde**
95e	**Güneysınır**	**wheat**	**3.75** ± 0.144 cde	**41.6*** ± **1.60 def**
107d	Seydişehir	wheat	3.50 ± 0.000 def	38.8 ± 0.00 efg
212d	Kadınhanı	barley	3.50 ± 0.000 def	30.0 ± 0.00 efg
230b	Selçuklu	wheat	3.50 ± 0.144 def	38,8 ± 1.60 efg
212d/1	Kadınhanı	barley	3.50 ± 0.000 def	38.8 ± 0.00 efg
206a	Sarayönü	wheat	3.25 ± 0.250 efg	36.1 ± 2.77 fgh
212x	Kadınhanı	barley	3.25 ± 0.144 efg	36.1 ± 1.60 fgh
233/1c	Selçuklu	wheat	3.25 ± 0.144 efg	36.1 ± 1.60 fgh
103b	Yalıhöyük	wheat	3.00 ± 0.000 fgh	33.3 ± 0.00 ghi
234e	Selçuklu	wheat	2.80 ± 0.115 gh	31.1 ± 1.28 hij
95c	Güneysınır	wheat	2.75 ± 0.144 ghi	30.5 ± 1.60 hijk
206g	Sarayönü	wheat	2.75 ± 0.144 ghi	30.5 ± 1.60 hijk
97b	Bozkır	wheat	2.60 ± 0.057 hij	28.8 ± 0.64 ijk
94/1d	Güneysınır	wheat	2.50 ± 0.000 hij	27.7 ± 0.00 ijkl
94/1b	Güneysınır	wheat	2.30 ± 0.057 ijk	25.5 ± 0.64 jkl
233a	Selçuklu	wheat	2.25 ± 0.144 jk	25.0 ± 1.60 jkl
103d	Yalıhöyük	wheat	2.20 ± 0.000 jk	24.4 ± 0.00 kl
94b	Güneysınır	wheat	2.00 ± 0.000 k	22.2 ± 0.00 l
211c	Kadınhanı	barley	2.00 ± 0.000 k	22.2 ± 0.00 l
211a	Kadınhanı	barley	2.00 ± 0.120 k	22.2 ± 1.33 l
95d	Güneysınır	wheat	1.40 ± 0.000 l	15.5 ± 0.00 m
95f	Güneysınır	wheat	1.10 ± 0.057 m	12.2 ± 0.64 m
Control (*F. culmorum*)	–	–	0.00 ± 0.000 n	00.0 ± 0.00 n

Note. Mean ± standard deviation; * – the pathogen inhibition efficiencies above 40%; in the columns, values having the same letter are not statistically different at $P < 0.05$ (Tukey HSD test).

The superior inhibition zones of isolates 213f, 233d and 219b are shown in Figure.

A successful antagonistic agent would be selected by *in vitro* screening as the first stage. El-Sayed et al. (2014) isolated native bacteria from rhizospheric arid soils and did the first selection based on their ability to inhibit *Fusarium oxysporum* and *Sclerotinia sclerotiorum* to evaluate for growth-promoting ability

Note. The control Petri dishes completely covered by *F. culmorum* are on the left and the effective isolates with superior inhibition zones provided by isolates 213f (A), 233d (B) and 219b (C) are on the right in the pictures; the arrows on the right Petri dishes indicate the inhibition zones resulting from the interaction between the pathogen and the isolate.

Figure. The *in vitro* antagonsitic activities of isolates 213f, 233d and 219b

and antagonistic potential against phytopathogenic fungi. The literature data indicated that various reasons for the inhibitory activity of bacteria could be enzymes, toxic metabolites that can damage the fungal growth via cellular walls (Wachowska et al., 2017).

ACC-deaminase activity. All of 31 isolates showing antifungal activity on the growth of pathogen on PDA were included in ACC-deaminase activity assay. All isolates with different degrees of efficacy for utilisation ACC as N source were divided into three groups on the basis of growth measured as optical density (OD). The OD_{600} values of ACC-utilization of isolates ranged from 0.161 to 0.974. As seen in Table 2, the isolates coded as Gu2 and 127b were grouped in group H with the highest growth (OD_{600} > 0.75). Three isolates 94b, 94/1b and 94/1d were in group M with medium growth (OD_{600} = 0.75–0.50), while the rest of isolates 219b, 233d, 213f, 95b, 123b, 127a, 130a, 95e, 107d, 230b, 212d, 206a, 212x, 233/1c, 103b, 234e, 95c, 206g, 212d/1, 97b, 233a, 103d, 211c, 211a, 95d and 95f were in group L with the lowest growth (OD_{600} < 0.50).

Table 2. Isolate groups based on their ACC-deaminase activity assay

Isolate	Group H	Group M	Group L
	OD_{600} > 0.75	OD_{600} = 0.75–0.50	OD_{600} < 0.50
219b			✓
233d			✓
213f			✓
127b*	✓		
95b			✓
123b			✓
127a			✓
Gu2*	✓		
130a			✓
95e			✓
107d			✓
230b			✓
212d			✓
206a			✓
212x			✓
233/1c			✓
103b			✓
234e			✓
95c			✓
206g			✓
212d/1			✓
97b			✓
94/1d		✓	
94/1b		✓	
233a			✓
103d			✓
94b		✓	
211c			✓
211a			✓
95d			✓
95f			✓

* – isolates with the highest optical density (OD_{600}) value

In order to screen the ACC-deaminase activity of bacterial isolates, this method has been successfully used by many researchers. Our results were in line with the results of other researchers. Saini and Khanna (2013) reported that a total of 9 rhizobacterial isolates belonging to genera *Bacillus* (6) and *Pseudomonas* (3), which were able to utilize ACC as the sole N source, their ACC-metabolizing rate measured as OD_{496} and OD_{600} ranged 0.54–1.39 and 0.165–1.120, respectively. Bal et al. (2013) isolated 355 bacteria from the rhizosphere of rice plants grown in the farmers' fields in the coastal rice field soil from five different locations of the Ganjam district, India. In their study, a bacterial isolate with the highest ACC-deaminase activity was grouped in *Bacillus* genera as a result of phylogenetic analysis. Jacobson et al. (1994) tested three *Pseudomonas* strains: *P. putida* GR12-2, *P. fluorescens* 34-13 and *P. aeruginosa* ATCC 10145, for the ability to utilize ACC as a sole N source on DF (Dworkin, Foster, 1958) salts minimal medium supplemented with ACC.

The researchers reported that only *P. putida* GR12-2 grew well on DF salts minimal medium supplemented with ACC. The researchers attributed the substantial growth of *P. putida* GR12-2 on the media with ACC to the fact that the strain possessed an enzyme able to hydrolyse ACC.

In vivo efficiencies and identification of rhizobacterial isolates. Two isolates Gu2 and 127b with the highest OD value in the ACC-deaminase activity assay were also tested for their effectiveness on suppression of *F. culmorum in vivo* conditions. The isolates had variable *in vivo* efficiencies for the pathogen suppression in pot trials under controlled conditions. In the trial, all pathogen-infected plants in both rhizobacterial contaminated and positive control pots were scored as 3 of scale value as seen in Table 3. Isolate Gu2 obtained from the wheat rhizosphere in Çumra district was the most effective one for suppression the pathogen infection on wheat seedlings with the efficiency percentage of 81.25 *in vivo* assay, while the both isolates highly succeed in suppression of the pathogen *in vitro*. The isolate 127b somewhat provided success with an efficiency percentage of 37.75 on *F. culmorum* infection of wheat seedlings. Statistically, there were significant differences (F = 230.495, df = 31, P = 0.001) among the isolate efficiencies under *in vivo* conditions. It was determined that both isolates belonged to *Bacillus* genera by MALDI Biotyper System classification.

The obtained results confirmed the presence of *Bacillus* spp. in the soil samples collected at different locations in Konya and Karaman provinces. Bacteria from the *Bacillus* group are microorganisms that inhabit a large number of different habitats. *Bacillus* are well known for their antagonistic effects and their ability to trigger inducing systemic resistance (Glick, 2014; Bjelic et al., 2018). The isolates of the genera primarily characterised by the ability to produce hydrolytic enzymes such as chitinases, glucanases, cellulases, lipases and proteases that prevent pathogen infection in the plant by demolishing the fungal and bacterial cell wall (Altınok et al., 2013). It is demonstrated that *Bacillus* isolates may inhibit the development of *F. graminearum* on wheat spikes and limit the production of DON mycotoxin (Palazzini et al., 2011). In their study, an isolate B 1 of *Bacillus* sp. had an antagonistic effect on three *Fusarium* species.

Nourozian et al. (2006) reported that *Bacillus subtilis* isolates were potent antagonists of *F. graminearum* and pointed out that their metabolites had a much stronger fungicidal effect inhibiting the

Table 3. *In vivo* assay results of the isolates Gu2 and 127b with the highest ACC-deaminase activity on wheat seedlings for suppressing of *Fusarium culmorum*

Isolate	Number of healthy plants	Number of diseased plants	Scale value	Disease severity %	*In vivo* efficiency %
Gu2*	**13**	**3**	**3**	**18.75**	**81.25 ± 10.07 a**
127b	6	10	3	62.50	37.50 ± 12.50 b
Positive control (*F. culmorum*)	0	16	3	100.00	0.00 ± 0.00 c
Negative control (sterile distilled water)	16	0	0	0.00	100.00 ± 0.00 a

Note. * – isolate that provided the highest pathogen control with the efficiency of 81.25%; in the columns, values having the same letter are not statistically different $P < 0.05$ (Tukey HSD test).

growth of its colonies by 97% in comparison with the control. The results presented in this study indicate that a rhizobacterial isolate Gu2 belonging to *Bacillus* genera with ACC-deaminase activity has potential as bio-control agent against *F. culmorum*. On the way to achieving a sturdy biocontrol agent, action mechanisms such as antimicrobial metabolite production of isolate Gu2 are necessary to study comprehensively to understand its role in the control of *F. culmorum.* Antimicrobial compound production of *Bacillus* species is widely known as one of the most important biocontrol mechanisms (Fan et al., 2017; Tan et al., 2019).

The wheat production is affected by *F. culmorum* as the disease severity is gradually increasing depending on the environmental factors (Liu, Liu, 2016; Streit et al., 2016). Control of such soil-borne disease might be possible with a well-featured biocontrol agent. In our study, a capable bacterial isolate belonging to *Bacillus* genera from the wheat rhizospheric soil with high biocontrol potential for wheat soil-borne pathogen *F. culmorum* and comprising ACC-deaminase activity was obtained. This multi-faceted isolate with ACC-deaminase enzyme activity might be useful in developing novel inoculants, leading to more efficient biocontrol strategies to cope with multiple plant stress factors. Glick (2014) emphasised that the key bacterial trait in promoting plant growth is the comprising of the enzyme ACC-deaminase and much more work in both basic and applied areas needs to be done. By this study, it was contributed to the point highlighted by the researcher.

The findings obtained from the study give us hope for developing a good biocontrol agent. Undoubtedly, further studies should be carried out with the successful isolate such as its effects under other plant stress factors, its mechanism of action, its survival in rhizosphere, its best inoculating method, its multiple stress combatant ability and combination with other microorganisms for an accomplished biocontrol agent.

Conclusions

1. In total, 215 soil samples were collected from the rhizosphere of cereal plants planted in all districts of Konya and Karaman provinces involved in intensive cereal production in Turkey. A total of 463 territorial rhizobacterial isolates showing morphological differences on nutrient agar were obtained from the soil samples.

2. Thirty one out of 463 isolates exhibited antifungal properties on *Fusarium culmorum* growth on PDA with inhibition zones ranging from 1.10 to 6.25 cm. Ten isolates: 219b, 233d, 213f, 127b, 95b, 123b, 127a, Gu2, 130a and 95e, out of 31 showing antifungal activity exhibited higher success with efficiencies above 40% to suppress *F. culmorum* growth *in vitro*. All isolates with *in vitro* antifungal activity were obtained from winter wheat rhizosphere excluding six isolates, which were isolated from barley rhizosphere.

3. All of 31 isolates showing *in vitro* inhibition activity on *F. culmorum* were assessed for their enzyme ACC-deaminase activity using ACC as the sole N source and grouped based on growth measured as optical density (OD). Among them, two isolates Gu2 and 127b were grouped in group H with the highest growth (OD_{600} > 0.75). Three isolates (94b, 94/1b and 94/1d) were in group M with medium growth (OD_{600} = 0.75–0.50), while the rest of isolates (219b, 233d, 213f, 95b, 123b, 127a, 130a, 95e, 107d, 230b, 212d, 206a, 212x, 233/1c, 103b, 234e, 95c, 206g, 212d/1, 97b, 233a, 103d, 211c, 211a, 95d and 95f) were in group L with the lowest growth (OD_{600} < 0.50).

4. Two isolates Gu2 and 127b with the highest OD value in the ACC-deaminase activity assay were also tested for their effectiveness on suppression of *F. culmorum* in wheat seedlings in controlled conditions. Both isolates Gu2 and 127b suppressed the pathogen infection on wheat seedlings with the efficiency percentages of 81.25 and 37.75 *in vivo* assay, respectively. It was determined that both belonged to *Bacillus* genera by MALDI Biotyper System classification proving the presence of *Bacillus* spp. in soil samples collected at different locations in Konya and Karaman provinces of Turkey.

5. The isolate Gu2 obtained from winter wheat rhizosphere in Çumra district exhibited high performance in the *in vitro* and *in vivo* suppression as well as ACC-deaminase activity assays. Apparently, its high performance in the control of *F. culmorum* and ACC-deaminase activity makes it promising towards becoming a novel biocontrol agent.

Acknowledgements

We are grateful to Prof. Dr. Ahmet Bayram for his valuable contributions to statistical analysis, and we would like to thank Mrs. Gülay Gürkan who worked in our team temporarily for her helpfulness and enthusiasm.

Received 05 04 2019
Accepted 17 01 2020

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ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 107, No. 2 (2020), p. 105–112
DOI 10.13080/z-a.2020.107.014

Rizosferos bakterijų, pasižyminčių fermento ACC-deaminazės aktyvumu, *Fusarium culmorum* biologinės kontrolės kviečiuose galimybės

G. Imriz[1], F. Özdemir[2], M. S. Karaca[2], M. N. Taş[2], İ. Topal[2], B. Ercan[2]

[1]Dicle universiteto Žemės ūkio fakultetas, Turkija
[2]Bahri Dağdaş tarptautinis žemės ūkio tyrimų institutas, Turkija

Santrauka

Fusarium culmorum (W.G. Smith) yra vienas iš grybinių dirvožemyje esančių augalų patogenų, dėl kurio patiriami nemaži javų derliaus ir kokybės nuostoliai. Tokiems patogenams kontroliuoti nepakanka vien cheminių priemonių, be to, jų taikymas yra žalingas aplinkai ir gyviems organizmams. Todėl chemines kontrolės priemones galėtų pakeisti aplinkai nekenkiantys, augalams naudingi mikroorganizmai, taip pat ir bakterijos. Daugelio kitų tyrimų duomenys atskleidė, kad kai kurie augalų augimą skatinančių rizobakterijų kamienai, turinys fermentą ACC (1-aminocyclopropan-1-karboksilate)-deaminazę, galėtų veikti kaip biologinės kontrolės priemonės, mažinančios perteklinį metileno kiekį augaluose, veikiamuose biotinio ir abiotinio streso arba abiejų stresų tuo pačiu metu. Siekiant nustatyti fermento ACC-deaminazės aktyvumą ir *F. culmorum* slopinimo *in vitro* / *in vivo* galimybes, tyrimo metu vertinti rizobakterijų izoliatai, gauti iš javų augalų rizosferos Turkijos Konya ir Karaman provincijose. Taikant dvigubos kultūros metodą iš 463 tirtų rizobakterinių izoliatų *F. culmorum* augimą ant bulvių dekstrozės agaro *in vitro* sėkmingai slopino 31 izoliatas. Po to efektyvūs izoliatai buvo tirti dėl fermento ACC-deaminazės aktyvumo, naudojant ACC kaip vienintelį azoto (N) šaltinį. Siekiant įvertinti *in vivo* patogenų slopinimo galimybes kviečių daiguose, du izoliatai Gu2 ir 127b, pasižymintys didžiausiu fermento ACC-deaminazės aktyvumu, buvo tirti vegetaciniuose bandymuose kontroliuojamomis sąlygomis. Izoliatų Gu2 ir 127b patogenų slopinimo efektyvumas *in vivo* buvo atitinkamai 81,25 ir 37,50 %. Remiantis MALDI Biotyper sistemos klasifikavimo rezultatais nustatyta, kad abu rizobiniai izoliatai priklauso *Bacillus* spp.

Reikšminiai žodžiai: dirvožemis, fermentas, kontrolė, kviečiai, patogenas, rizobakterijos.