© by PSP Volume 25 – No. 9/2016, pages 3493-3506 Fresenius Environmental Bulletin

BIODEGRADATION OF ESSENTIAL OIL BY *ENTEROBACTER* SP. INDUCES PLANT GROWTH AND INHIBITS DAMPING-OFF PATHOGENS

Ragip Soner Silme[1], Omur Baysal[2*]

[1] Istanbul University Scientific Research Projects, Istanbul University Main Campus Beyazit, Fatih 34452, Istanbul, Turkey
[2] Muğla Sıtkı Koçman University, Faculty of Science, Department of Molecular Biology and Genetics 48000 Muğla, Turkey.

ABSTRACT

The soil microflora shelters community of dynamic microorganisms affecting biological activities of plant pathogens. Essential oil of *Thymbra spicata* oil is known to have antibacterial property. However, *Enterobacter* sp., which is able to decompose essential oil of *Thymbra spicata* and its main component carvacrol, was identified using 16S rDNA analysis. To elucidate this inhibitory effect, antifungal properties of secreted metabolites were investigated using bioautography of culture filtrates production of indole acetic acid (IAA) and chitinase. Chitinase expression was confirmed using SDS-PAGE staining by protein extraction from culture mediums. Therefore, we detected that biodegraded metabolites have also plant promoting effect on plants and inhibitory effect to *Fusarium oxysporum* and *Rhizoctonia solani*. These findings suggested that *Enterobacter* sp. is an IAA and chitinase producer that induces plant growth and biological agent to soil-borne pathogens.

KEYWORDS:
Assimilation, biodegradation, plant growth regulators, plant pathogens

INTRODUCTION

A number of essential oil constituents have inhibitory effect on plant pathogens [1]. Many studies on microorganisms able to assimilate of essential oils that have been already published [2], but these studies remained in a limited area that could not intensively be reflected to scientific area and not integrated into in practice [3, 4].

Recycling of chitin from disposed materials and dead organisms result mainly from the activity of chitinolytic microorganisms. Species of the genera *Serratia*, *Bacillus*, and *Vibrio* have been reported to secrete several chitinolytic enzymes and chitin-binding proteins, which are thought to degrade chitin synergistically, into the extra-cellular environment [5, 6, 7, 8]. The volatile oils of some conifers also contain carvacrol and thymol [9]. It has been indicated that the antioxidant activity of the essential oils of the above-mentioned herbs are due to the carvacrol, its isomer thymol and some other phenols [10, 11]. Chamberlain and Dagley [12] found that *Pseudomonas* spp. strains were able to degrade thymol and carvacrol. They proposed also a metabolic pathway for thymol involving meta-ring opening of a trihydric phenol, 3-hydroxythymo-1,4-quinol to 3,7-di-methyl-2,4,6-trioxo-octanoate. The hydrolysis of the latter, catalyzed by-ketolase yields acetate, 2-ketobutyrate and isobutyrate. -ketolases encompass ten known enzymes that hydrolyze carbon-carbon bonds of -diketo compounds. The selective antimicrobial effect of EO vapour on microorganism population results surviving micro-flora profiles, which induces decomposing of the essential oil as carbon source and producing different metabolites.

The most common soil-borne pathogens infecting young seedlings in the greenhouses are *Rhizoctonia solani* and *Fusarium moniliforme* causing damping-off disease in Turkey. There is no sufficiently effective pesticide to control these pathogens. In the present study our findings showed decomposition of the essential oil and its major components, which can be associated with the superiority of the *Enterobacteriaceae* family members.

To the best of our knowledge, there is no information on bacterial biodegradation of the essential oil and growth promoter effect of its degraded metabolites on crops. In this study, the effect of metabolites at post-degradation process considering two different concepts including its plant growth promoting rhizobacteria effect (PGPR) and fungitoxic effects on soil-borne pathogens were examined.

MATERIALS AND METHODS

Bacterial isolation. Bacteria was isolated from the endorhizosphere soil of *Thymbra spicata* var. *spiciata* plants and performed in a solid and liquid culture medium (A) (which only carbon sources modified from King et al. [13]) containing 50, 100, 250 and 500 ppm essential oil of *T. spicata* besides peptone 20g, K_2SO_4 10g, $MgCl_2$ 1.4g, glycerol 10ml L^{-1}, agar 13.6 in distilled water 1000mL pH 7.2 at 25ºC for 48 hours *in vitro* conditions. A colony able to grow in a medium containing 500 ppm EO [14, 15, 16, 17] was detected and further studies was carried out on this colony.

Bacterial identification. To identify the bacterium, a polymerase chain reaction (PCR) was performed to amplify the 16S rRNA gene from the genomic DNA of strain Enterobacter as described in reference. The forward primer was 5'-TGGCTCAGAACGAACGCTGGCGGC-3', and the reverse primer was 5'CCCACTGCCTCCCGTAAGGAGT-3. The temperature cycle was at 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min 30 s for 30 cycles and 5 min at 72°C for extension. The PCR product was cloned in a pGEM-T easy vector (Promega, Madison, WI, USA). The nucleotide sequence of the 16S rRNA gene was determined by an ABI Prism 377 DNA sequencer (PE Applied Biosystems, MA, USA) and compared with published 16S rRNA sequences using Blast search at Genbank data base of NCBI (Bethesda, MD, USA). Our results were also confirmed with the results of Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

Fungal plant pathogens. Tested soil-borne fungal plant pathogens *Rhizoctonia solani* Kühn and *Fusarium moniliforme* Sheldon were provided from previous pathogen collections [9].

Assessing of bacterial assimilation. The *Enterobacter* sp. was inoculated to 3 different liquid medium A to evaluate its essential oil's assimilation capacity. Therefore, ingredients of culture medium A were different according to carbon source. These A mediums contained glycerol 10 ml L^{-1} alone, *T. spicata* essential oil (extracted from previous studies [9]) (500mg L^{-1}) and carvacrol (%98, Sigma–Aldrich, CAS Number: 499-75-2) 500mg L^{-1} seperately. The suspension of bacterium was adjusted to ca. $4x10^9$ colony forming units (CFU) ml^{-1}, of suspension 1 ml was inoculated into each 125 ml flask and was grown in shaking culture (105 rpm) for 6 days at 25ºC. There was no bacterial suspension in control flasks. Bacteria were performed in media (40 ml per each medium type) up to 2, 4 and 6 days after inoculation.

Detection of indole-3-acetic acid production using different carbon sources and PGPR effect of bacteria. The method by Torres-Rubio et al. [18] was used for detection and to estimate of indole-3-acetic acid (IAA) production. Essential oil, glycerol and carvacrol were used to be carbon sources in culture mediums. Control mediums were not inoculated. 5 ml of bacterial suspension was separated from liquid medium at 48, 96, and 144 h after inoculation hours. The suspension was centrifuged at 9500 rpm for 20 min at 5° to discard pellets. After 30 minutes, aliquot was measured by spectrophotometer (Shimadzu UV-160 A) at 530 nm after mixing with Salkowski reagent (2:1) indicating pink color according to colour change. Concentrations were calculated according to plotted calibration curve. The bacterial suspension was adjusted to $2.7x10^8$ CFU ml^{-1}. Furthermore, another pot experiment was conducted to investigate whether bacteria had any PGPR effect on roots.

Observing plant height and root mass enhancement with in vivo experiments. As another marker of IAA production, remarkable increasing of plant height and root mass enhancement were visually observed in another experiments conducted with cucumber (*Cucumis sativus*) seedlings compared to control under *in vivo* conditions. Cucumber seeds were sown into a mixture of soil, peat soil and perlit (2:2:1). The soil was not sterilized to protect the microbial flora. Bacteria were grown as described in Assessing of Bacterial Assimilation. Bacteria inoculated liquid culture medium with essential oil was drenched to plants through the roots, 144 hours later inoculation. Water alone was control. They were introduced to plants by 1/1000 dilution with water. Plants were grown in 26±ºC with relative humidity 60% ±5 and under 14 hours day light. Twenty days later, 10 plants were randomly selected and fresh and dry weight of the plants (fresh / dry leaf weight, fresh / dry root weight, root length, and leaf area index) were measured. Leaf area index were measured using a software of Comparative Plant Ecology The Unit in Sheffield University. The data was statistically evaluated using Duncan test [19].

Detection of post-degradation metabolites and their fungitoxic effect. Extraction was done according to Burkhead et al. [20], adjusting pH to 3 with diluted HCI in liquid mediums. Of mediums 8 ml sample was pipetted into glass screw-capped tubes and 1.5 ml ethyl acetate was added into tubes mixed thoroughly with vortex. Then extracts were centrifuged at 9500 rpm for 20 min at 5ºC. 400 µl extract was spotted onto aluminum thin layer

chromatography (TLC) sheets (Kieselgel 60 F_{254} 20x20 cm). The plates were developed in TLC tank containing 100 ml chloroform: acetone (9:1) [20], and were allowed to develop until the solvent, which was 15 cm above the loading point. The plates were immediately marked with a pencil. Then the plate was allowed to dry. When the dried plates were viewed under UV radiation (254nm), *Rf* values of the spots were determined. The bioautography method was used spraying mixture of dextrose and spores of *F. moniliforme* Sheldon grown in PDA for 7 days at 24 ^{0}C to detect the fungitoxic compounds [20]. Bioautography method was made with three replicates to validate the antimicrobial substance.

Inhibitory effect of post degradation products on soil-borne pathogens. The liquid A mediums were tested to assess the inhibitory compounds in assimilation products to soil pathogens *R. solani*, *F. moniliforme*. The bacterium was performed in liquid A medium (pH 7.2) containing different carbon sources that were essential oil and essential oil+glycerol seperately. The inoculated mediums were grown using a rotary shaker for a week (25ºC, 105 rpm min^{-1}). Of liquid mediums, 266 ml was mixed with sterilized 734 ml PDA, and the total amount was 1000 ml. Of the flasks 15 ml mixture was dispersed into agar medium and poured into plates. Disks with (10 mm diameter) mycelium of performed pathogens *Rhizoctonia solani* Kühn, *Fusarium moniliforme* Sheldon were released onto center of petri dishes and performed for 3 days in 27ºC. Of the same solid mediums 15 mL was used in *in vitro* assays. The control mediums contained no bacterial filtrate. Moreover, the inhibitory effect of bacterium on soil borne pathogens, which can be associated with secondary (degraded) metabolites, was also investigated using liquid A mediums (pH 7.2) containing different carbon sources. At this time, bacterium was performed in liquid media (pH 7.2) containing no glucose, 2% glucose, and 0.1% glucose+500 ppm *T. spicata* oil separately. These mediums were also introduced adjusting three different ratios ((5ml; 15ml)1/4, (10ml:10ml) 1/2, (15ml:5ml) 3/4). Of the flasks totally 20 ml mixture was dispersed into agar medium and poured into plates. All plate experiments were carried out with 5 replicates and measurements were done perpendicular to the side of fungus-mycelium growth as given above. The data was recorded at 7 days after inoculation. The experiments were carried out according to Completely Randomized Design, Duncan test was done for evaluations of the mean value differences ($P<0.05$) [19].

Detection of chitinase activity of bacteria in different eediums. Essential oil of *T.spicata* (500 mg L^{-1}), carvacrol (500 mg L^{-1}), glycerol (10 ml L^{-1}) and glycerol+carvacrol (5 ml L^{-1} glycerol and 250 mg L^{-1} carvacrol) were separately used in medium A as carbon sources. Control groups were not inoculated with bacteria. Of inoculated mediums, samples were collected at 48, 96, 154 and 240 h after inoculation, pH and chitinase activity were measured. Chitinase activity measurement was done according to Isaacson and Webster's [21]. The activity in culture mediums was measured on the basis of the release of p-nitrophenol from labeled substrates. Testing chitinase activity, in 50-mM potassium phosphate buffer, 1-mM substrate solution was dissolved with *p*-nitrophenyl-N-acetyl-β-D-glucosaminide (0.34 mg/ml) (pH 6.7). All the measurements were done using an Elisa plate reader (Thermo Labsystems Multiskan Spectrum) at 410 nm after incubation for 120 min at 41˚C. Chitinase activity was measured both in samples containing bacteria and after removing bacteria by centrifuge of liquid media.

Staining of chitinase activity on SDS-Page electrophoresis. Prepared SDS gel (BIO-Rad, 10 wells) was used. Electrophoresis was performed at 60 V in the first 2 h, then at 110 V for 1 h more, after which the gel was removed from the electrophoresis tank. When SDS-PAGE electrophoresis was finished, one was fixed and stained with CBB R-250 dye (2.5.1). The other was immersed and shaken twice in 25% (V/V) isopropanol in 10 mM acetate buffer (pH 5.0) for 10 min, and equilibrated in 10 mM acetate buffer (pH 5.0) for 15 min before activity staining. The activity staining of chitinase was mainly according to the method of Trudel and Asselin [22]. The 1 % (W/V) agarose gel (pH 5.0) containing 0.01% (W/V) deacetyl glycol chitin was overlaid with the equilibrated polyacrylamide gel. After incubation at 37°C for 1 h, the agarose gel was dipped in 0.01% (W/V) Fluorescent brightener 28 (designated as Calcofluor white M2R in the original paper) fluorescent dye (F-3543, Sigma Chemical Co.) in 50 mM Tris buffer (pH 8.3) for 5 min and then washed with distilled water several times to remove excess fluorescent dye. The active chitinase in an agarose gel appeared dark on a bright background under UV light.

Protein patterns. Prepared SDS gel (Fa. BIO-Rad, 10 wells) was used for determination of protein patterns. The electrophoresis tank was filled with 1% SDS puffer (Roth Co.). Combs were removed from stacking gel, taking care not to disturb the well dividers. The samples (5 µl) and standard protein as marker (low molecular weight–marker, Pharmacia calibration Kit; 14.4, 20.1, 30, 43, 67, and 94 kD were applied in 1:1 (v/v) sample buffer in well dividers.

Staining of gel with Coomassie Brilliant Blue. After the run, the gel was placed into coomassie solution (0.25 g Coomassie – blue, 400 ml methanol 70 ml glacial acetic acid, 1 l distilled water) since proteins are not directly visible and incubated for 1 h at room temperature. Coomassie blue dye binds to protein non-specifically. Corresponding protein bands can be detected as blue bands on a clear background [23]. Thereafter, the gel was placed in the destaining solution (100 ml isopropanol, 100 ml glacial acetic acid, and 800 ml distilled water) under gentle shaking for 4 h to remove the background, prior to evaluation and photography. Protein electropherograms of samples were compared visually with the marker. After removing the solution, the bands of visible protein bands were fixed with 50% glycerol solution and stored in a refrigerator.

RESULTS

Identification of bacteria. Identification of bacterium was made using 16S rDNA analysis and determined as *Enterobacter* sp. 16S rRNA gene sequence showed also high similarity (98-100%) to *Enterobacter* sp. according to BLASTn program.

Detection of indole-3-acetic acid production using different carbon sources and PGPR effect of bacteria. Uninoculated mediums failed to produce any IAA (Table 1). IAA was assessed in inoculated A mediums except for the ones containing glycerol since bacteria did not produce IAA in the mediums containing glycerol alone to be a carbon source. IAA quantity was 19.2 µg ml^{-1} in the medium containing carvacrol alone (Table 1). The medium containing only essential oil IAA quantity was nearly equal amount as detected in medium containing carvacrol that was 19.7 µg ml^{-1} (Table 1).

Observing plant height and root mass enhancement with in vivo experiments. In sterilised soil, there was significant difference on plant heights between control and inoculated A medium ($P<0.05$). The highest fresh weight was in plants treated with inoculated A medium (Table 2). The difference was also noticeable in view of root weight and fresh weight, leaf area index within treatments (Table 2). The green leaf weight was 332.32 mg/plant for the application of inoculated medium containing essential oil and 189.63 mg/plant for the tap water. This difference is indicative for growth promotive effect.

TABLE 1
IAA (µg/ mL) production by bacteria performed using different carbon sources according to hours post inoculation.

Time (hour)	EB	GB	CB	Econt	GCont	CCont
48	10.7±0.4	0	12.1±0.8	0	0	0
96	20.4±1.6	0	20.5±2.2	0	0	0
144	19.7±1.3	0	19.2±1.3	0	0	0

EB; bacteria performed in medium A containing essential oil alone,
GB; bacteria performed in medium A containing glycerine alone,
CB; bacteria performed in medium A containing carvacrol alone,
Econt; medium A containing essential oil alone,
Gcont; medium A containing glycerine alone,
Ccont; medium A containing carvacrol alone.

TABLE 2
The statistical analysis after application of inoculated medium containing essential oil (EB) and control group (tap water) on cucumber plant (sd=standart deviation). * in same row with different letters show statistically difference according to Duncan test (P<0.05).

*Measured properties	EB±sd	Control±sd
Green plant weight (mg/plant)	1930 ± 330^{A}	1160 ± 150^{B}
Green leaf weight (mg/plant)	332.32 ± 37^{A}	189.63 ± 34^{B}
Dry leaf weight (mg/plant)	35.89 ± 80^{A}	24.11 ± 5^{B}
Green root weight (mg/plant)	92.78 ± 41^{A}	29.88 ± 8^{B}
Dry root weight (mg/plant)	16.02 ± 4.9^{A}	8.96 ± 2.2^{B}
Root length (mm/plant)	144.6 ± 45.9^{A}	99.6 ± 31.2^{B}
Plant area measurement (cm^2/leaf)	2.945 ± 0.34^{A}	1.73 ± 0.27^{B}

Detection of post-degradation products and their fungitoxic effect. Six days of inoculation was optimum time for detecting effective compounds after performing bacteria in A medium, which was determined with previously studies [14]. The samples after performing were subjected to TLC sheets. There was no similar zone in samples of uninoculated mediums, however, the effective compounds in different liquid mediums containing essential oil, glycerol and carvacrol resulted in appearance of the spots on TLC sheets at 0.32, 0.37 and 0.31 *Rf* values under UV radiation (254 nm). Carvacrol appeared both in inoculated and uninoculated TLC sheets at *Rf* 0.8 in. Inhibition zones that appeared around the spots remarkably inhibited fungal growth after its performing in liquid culture with different carbon sources containing essential oil, glycerol and carvacrol. The spots were identically same in terms of *Rf* values at around of 0.47, 0.45 and 0.49 under UV radiation (254 nm). Carvacrol portions extracted through inoculated and uninoculted mediums showed inhibition zones at *Rf* 0.8.

Inhibitory effect of post-degradation assimilation products on soil-borne pathogens. Our results showed less growth of *R. solani* on inoculated mediums containing essential oil compared to control. Inhibition was determined in inoculated mediums containing essential oil and glycerol. The effect of bacteria was statistically significant at 0.05 level compared to control (Table 3). The effect of bacteria against *F. moniliforme* was investigated, inoculated mediums gave rise to statistically significant (P<0.05) inhibition, which was compared to control. Inoculated mediums containing both essential oil and glycerol showed the highest inhibition zone respectively when compared with each other. Medium containing essential oil alone had the highest inhibition effect within controls (Table 3). The mediums introduced by adjusting three different ratios (1/4, 1/2, 3/4) showed similar results as given Figs 1, 2. Especially inoculated mediums showed the highest inhibition zones compared to uninoculated ones. *R. solani* and *F. moniliforme* showed less growth in inoculated mediums containing 0.1% glucose + 500 ppm essential oil than control plates (Fig. 1-2). Moreover, a recession in growth of mycelium shows some antimicrobial compounds production in inoculated mediums with %2 glucose in all ratios.

TABLE 3
Growth of the pathogens on plates containing different carbon sources. EB; bacteria was perfomed in a medium containing essential oil, GB; bacteria was performed in a medium containing glycerine, EGB; bacteria was perfomed in a medium containing both essential oil and glycerine. Ce; no bacteria but medium contains essential oil alone, Cg; no bacteria but medium contains glycerine alone, Cc; no bacteria but medium contains carvacrol alone. Control; PDA medium alone (± =standart deviation).

Tested pathogens	Mycelium diameters (mm) of the pathogens on plates according to different carbon sources						
	EB	GB	EGB	Ce	Cg	Cc	Control
R.solani	22.2±7.45 ^{D}	29.0±4.73 ^{CD}	0 ^{E}	33.7±3.89 ^{C}	76.6±0.89 ^{A}	51.8±11.97 ^{B}	77.0 ^{A}
F.moniliforme	6.6±9.03 ^{C}	2.2±1.48 ^{C}	0 ^{C}	24.3±8.32 ^{B}	52.5±3.80 ^{A}	47.5±8.40 ^{A}	43.6±8.24 ^{A}

*Groups in same row with different letter show statistically differences (P<0.05) according to Duncan test.

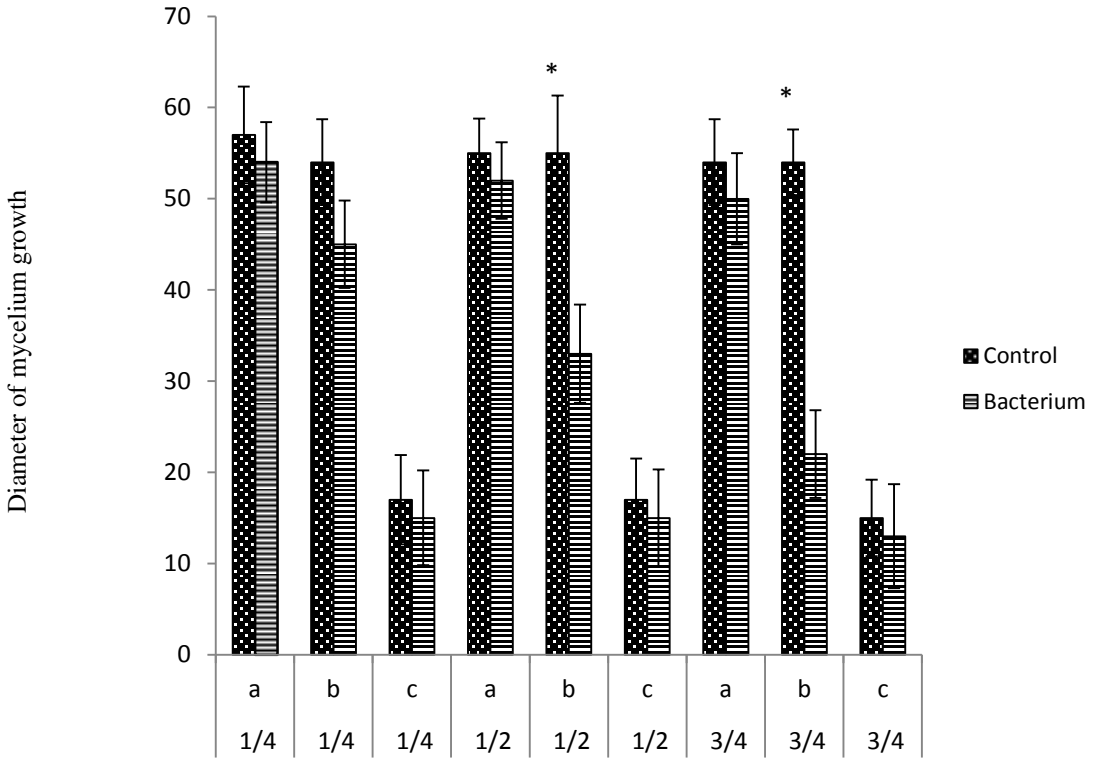

FIGURE 1
The effect of bacterium filtrate against *R. solani* (a; bacteria was performed in A medium containing no glucose (control), b; 2% glucose, c; 0.1% glucose + 500 ppm essential oil respectively). *Astericks show statistically differences (P<0.05) compared to control groups according to Duncan test.

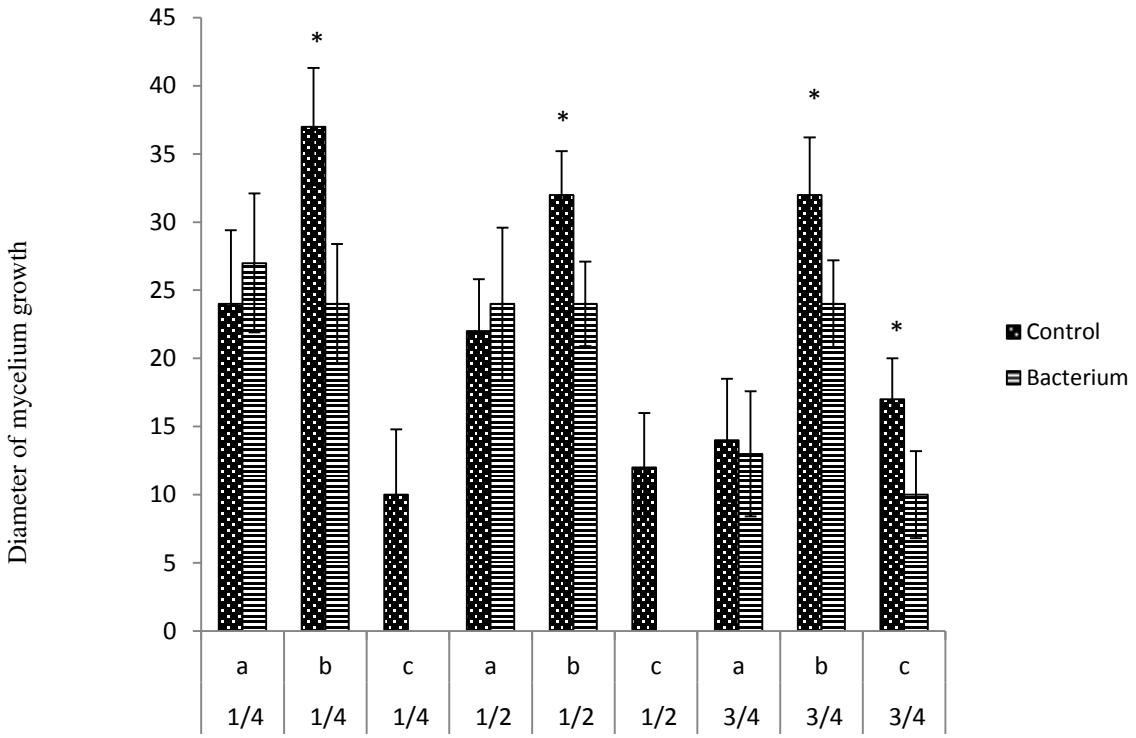

FIGURE 2
The effect of bacterium filtrate against *F. moniliforme*. (a; bacteria was performed in A medium containing no glucose (control), b; 2% glucose, c; 0.1% glucose + 500 ppm essential oil respectively).*Astericks show statistically differences compared to control groups (P<0.05) according to Duncan test.

Detection of Chitinase Activity of Bacterium in Different Mediums. None of the control groups showed chitinase activity in the experimental period. The pH of all mediums increased in the mediums except medium containing glycerol alone. This case can be associated with differentiation of bacterial metabolism by different carbon sources. The highest chitinase activity was always in inoculated mediums with glycerol and carvacrol at all hpi (Table 4). Later on, the highest activity was observed in inoculated medium containing essential oil (4.64) and inoculated medium with carvacrol (4.54) respectively. Inoculated mediums except for mediums containing glycerol alone showed chitinase production, but mediums containing no bacteria showed the lowest chitinase activity in the experimental period. Although no chitinase activity was detected in medium containing glycerol, inoculated mediums showed a gradual increase at 154 and 240 hpi in all mediums. This result indicates attacking of bacteria to pre-exist chitin in the medium. This was confirmed by no detected chitinase activity in medium containing glycerol when bacteria were removed from the medium (Table 4). The chitinase production of bacterium was associated with the observed inhibitory effect on tested pathogens. *In vitro* test results showed accordance with chitinase activity measurements. pH values of the inoculated medium A showed changes according to carbon sources. pH values in inoculated medium A containing glycerol alone were less than inoculated medium containing essential oil or carvacrol (Table 4).

TABLE 4
Changes in chitinase activity of mediums containing living bacteria and different ingredients. Hours post inoculation (HPI) and carbon sources are listed on left column. Bacteria; contains only bacterial suspension without culture medium. Control Medium; contains only culture medium, bacteria have been removed after performing from culture by centrifuge. Bacteria+Medium; it contains bacteria and culture medium, Gly+Carvacrol; medium contains glycerol and carvacrol, pH; pH values of the inoculated mediums ($2x10^6$ CFU ml^{-1}).

HPI	Carbon Sources	Bacteria	Control Medium	Bacteria + Medium	pH
48	Essential oil	1.88	0.36	2.27	8,07
	Glycerol	1.47	0,02	2.05	**5,31**
	Carvacrol	1.29	0,50	1.77	8,11
	Gly+Carva	2.45	0,24	**2.83**	6,85
96	Essential oil	1.48	2.06	**3.73**	8,60
	Glycerol	**1.39**	0,06	1.34	**4,66**
	Carvacrol	1.04	1.80	**3.14**	8,77
	Gly+Carva	1.95	3.98	**5.12**	8,50
154	Essential oil	1.27	1.98	3.88	9,33
	Glycerol	**2.10**	0,13	2.05	**4,73**
	Carvacrol	0,88	2.32	3.35	9,34
	Gly+Carva	2.22	4.68	**6.79**	9,15
240	Essential oil	1.51	2.40	**4.64**	9,48
	Glycerol	2.66	0.28	2.48	**4,72**
	Carvacrol	1.08	2.84	**4.54**	9,41
	Gly+Carva	**2.71**	**5.09**	**7.64**	9,52

SDS-Page Electrophoresis Results of Chitinase Activity. In SDS Page electrophoresis studies in uninoculated mediums, there was no chitinase expression. At 48 h of treatment the chitinase expression was detected on around of 30 kD and 43kD protein (Fig. 3). At the 2nd day of treatment the chitinase expresion was higher in two bands from protein bands when bacteria was incubated in medum containing essential oil, cavracrol and glycerol (Fig. 3), The protein bands were confirmed the heights of expressed proteins on SDS- PAGE Fig 4.

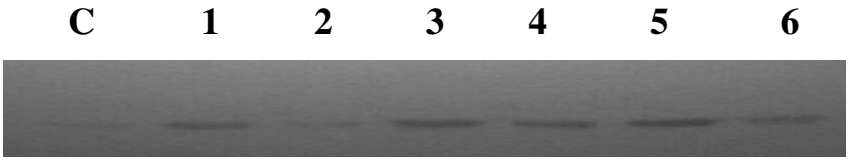

FIGURE 3
The confirmation of chitinase expression using SDS-Page electrophoresis
The active chitinase in an agarose gel appeared dark on a bright background under UV light. C. The chitinase expression in uninoculated and without carbon source mediums
1. The chitinase expression in culture medium containing essential oil (bacteria inoculated) at 48 hpi
2. The chitinase expression in culture medium containing essential oil (uninoculated) at 48 hpi.
3. The chitinase expression in culture medium containing essential oil at 48 hpi (bacteria inoculated)
4. The chitinase expression in culture medium containing cavracrol at 48 hpi (bacteria inoculated)
5. The chitinase expression in culture medium containing glycerol+essential oil at 48 hpi. (bacteria inoculated)
6. The chitinase expression in culture medium containing glycerol at 48 hpi (bacteria inoculated)

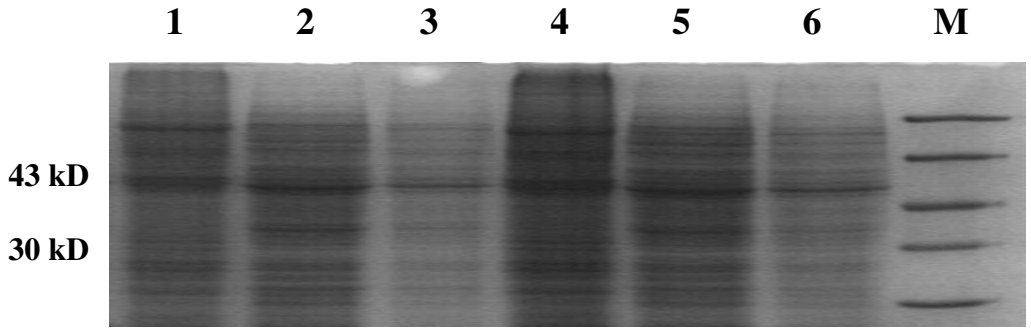

FIGURE 4
The protein patterns on SDS-Page gel electrophoresis
SDS gel electrophoresis was performed using a vertical mini- prepared gel (Bio-Rad Inc.) . The gel was fixed with 12.5% TCA and stained with CBB R-250 dye. M. Marker C. The protein pattern in untreated plants

DISCUSSION

In nature, a concentration gradient of plant volatile organic compounds is present from the releasing plant part to its environment. Essential oils vary in their chemical compositions containing only monoterpenes compared to ones with a large amount of monoterpenoids [24]. We selected essential oils of *Thymbra spicata* var. *spicata* and carvacrol as major compounds of it. Little information is known about the biodegradation of this volatile components. As known all the components are degradeable by microorganisms. Carvacrol, a component of *Thymbra spicata* L. var. *spicata* oil, is also degradable [2]. *Pseudomonas* sp., *Bacillus* sp., *Alcaligenes* sp. and many microorganisms are capable of utilizing terpenes or monoterpenes [25, 26]. However, this is the first and original finding on *Enterobacter* sp. using carvacrol as its carbon source. The *Enterobacter* sp. species was identified using 16S rDNA sequence according to different databases [27]. In our study the same method was employed and our isolate was identified to be *Enterobacter* sp. It is known, on *Enterobacter* sp. that its ability on utilizing of a wide range of sugars may contribute to its success for adaptation to roots of different plants. Plants are known to exude a wide range of substances [28]. The generally, the *Enterobacter* genus is one of the most common genera of bacteria isolated as plant endorhizosphere bacteria. It has been reported to be endorhizosphere bacteria in maize, rice, cotton, cucumber, common bean, broccoli and sweet potato [29, 30]. Furthermore the efficiency of PGPR is critically dependent on the tolerance ability under saline conditions in many soils, as well as it displays resistance to numerous antibiotics competing in microbial flora, *Enterobacter* sp. was also shown to tolerate 3% NaCl as well as resistant to inhibition by the streptomycin in another study [30]. The production of ammonia is frequently reported for PGPR, a process most probably resulting from the deamination (ammonification) of the amino acids present in the peptone which is used for this assay [31]. It has been suggested that ammonia may have a role in antagonism against competing flora, particularly the fungi [30, 32]. In our study, an *Enterobacter* sp. was also demonstrated to be a microorganism with very good potential for the promotion of plant growth. Lack of its host specificity is a good attribute for microbial inocula, which may be used for the cultivation of a wide range of crops. Zakria et al. [31] reported that another strain of *Enterobacter* sp. (strain 35) isolated from sugarcane has a successfully ability to colonize and promotion on the growth of *Brassica oleracea* (broccoli), a dicot. Endophytes offer a wide range of benefits to plants by promoting growth via several mechanisms, such as the production and regulation of levels of phytohormones, including IAA and ethylene [33, 34]. Since our isolate is a member of *Enterobacteriacea* family, the same properties can be suggested.

The high level production of the plant growth hormone (auxin), IAA *in vitro* is noteworthy. Recent findings have revealed that auxin biosynthesis plays essential roles in many developmental processes in plants including gametogenesis, embryogenesis, seedling growth, vascular patterning, and flower development [35]. IAA production is a major tool employed by PGPR. IAA is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR [4, 36, 37, 38]. The observed PGPR effect of our strain on plant growth can be correlated well

with the IAA production. Moreover, IAA has fungitoxic property on plant pathogens besides its PGPR effect [39, 40]. The secretation quantitiy of IAA plays crictical role on inhibiton of pathogen growth [41].

Bioautography is a useful technique in detection of the inhibitory compounds [42]. The bioautographic findings showed identical compounds having antimicrobial properties of *Enterobacter* sp. after biodegradation of essential oil. Directly spraying of conidial suspensions onto TLC plates has been an easy technique for detection of fungitoxic compounds [43, 44]. Our study showed a bacterial biodegradation, which can be associated with production of antimicrobial compounds released after assimilation of essential oil. This case was also proved with bioautography on bacterial metabolites containing IAA and chitinase.

The studies carried out on petri assays using *Enterobacter* sp. performed in medium containing essential oil, carvacrol and/or glycerol resulted in product changes depending on the carbon source during the bacterial biodegradation. Bioactive compounds were only detected with essential oil or carvacrol. Particularly, a correlation can be assumed within essential oil and/or carvacrol with IAA and chitinase production. Chitin is backbone of fungi cells providing rigidity and structural support to the thin cells of the microorganism. Chitinase is a chitin-degrading enzyme, which is an important tool for the resistance in bacterial invasion against fungus. Our data represents chitinase production associated with essential oil and carvacrol biodegradation. In the studies, glycerol was preferred due its prominent property for industrial usage and its biodegradation in environment. In another study it has also been used detecting and isolating a bacterium capable of metabolizing glycerol trinitrate (GTN) which is highly explosive compound [45]. Correspondingly, on our *Enterobacter* sp. the same capabilities may be suggested. However, the highest production of chitinase enzyme was observed in medium containing glycerol and carvacrol. This case was clearly confirmed by SDS-PAGE electrophoresis labelling. As a carbon source this case can be associated with the choice of the bacteria since glycerol is easily degradable compound. The raising of acidity level in medium containing glycerol can be related to converting ethanol by oxidative decomposition, which is a reason for pH decreasing in medium [46]. In assessing the inhibitory effect of *Enterobacter* sp. resulting from secondary metabolites, 0.1% glucose was also tested with 500 ppm essential oil to enhance the viability chance of the bacterial to be a imperative carbon source [47] before exposing to high concentrated essential oil (without any carbon source preference). These factors include host specifity, the population dynamics and pattern of host colonization, the movement within host tissues, and the ability to induce resistance.

It can be synergistic effect of carbon sources used together on the metabolites produced by bacteria. Preliminary studies gives some predictions on the metabolic products when carbon source is glucose or terpenoid compounds. Many researchers indicated that several species of the *Enterobacteriaceae* glucose is oxidized to gluconate and to 2-ketogluconate [48, 49, 50, 51] and also reducing compounds can be produced from gluconate [52, 53]. Also a few bacterial species, including *Acetobacter melanogenum*, *Gluconobacter oxydans*, and *Janthinobacterium lividum*, have showed production of 2,5-diketogluconate as an end product after glucose oxidation [54, 55, 56]. Decomposing of several terpenoid compounds, including R-carvone, S-carvone, cumene, carvacrol, thymol, R-limonene, S-limonene, p-cymene, and trans-cinnamic acid, by *Arthrobacter* sp. strain has published [57] that these are commonly in plants such as citrus, juniper, oregano, thyme and other aromatic plants [58]. Though genus was different in previous studies, *Enterobacter* sp. showed also similar properties.

It is also detected that adjusting pH to 7.2 indicated changes due to the acidic property of the fatty acids in essential oil and carvacrol at the beginning of the biodegradation experiment. Chadwick and Burg [59] and Gehring et al. [60] also indicated that IAA causes a decrease in pH and an increase in the cytosolic Ca2+ concentration in all the tissues. So that, pH changing to basic level or increasing can be suggested on bacteria in growing phase [61]. Bacteria are known to be a microorganism maintaining near-neutral intracellular pH level [62], but this case is considerably different when the cell is subjected to an acidic environment [63]. Creating an optimum condition effort by itself can be assumed for bacteria, which seems to be a competence to survivor. On the other hand our data originaly represents the quite quantity of the IAA and chitinase productions per a bacterial colony since the CFU/ml at the beginning were measured. This data indirectly gives correlation between the IAA production and its effect on plant height by the bacteria. In another study, a common protozoa in soil *Acanthamoeba* sp. induced changes in root morphology of watercress (*Lepidium sativum* L.) seedlings, which was also correlated well with its hormonal effects and increased the proportion of IAA producing rhizosphere community [64]. Within phytohormones IAA may indirectly improve P acquisition by plants by increasing root growth [65].

CONCLUSION

Producing environmental friendly and non-toxic effective molecules using smart biologics is a desire of all researchers in the world and its positive reflection of them in practice related to agricultural implementations seems to be possible in near future [66]. Our study demonstrate that non-pathogenic bacteria belonging to *Enterobacter* sp. species can be used as strategic tool for preventing the spread of soil-borne pathogens and to promote plant growth; however, the mechanism of this preventative effect still needs to be clarified using advanced techniques in further studies.

ACKNOWLEDGEMENT

The authors wishes to thank emeritus Prof.Dr. Oktay Yeğen for laboratory facilities and support in Plant Protection Department of Akdeniz University, in Antalya, Turkey.

Both authors have equal contributions on the study.

The Authors have declared no conflict of interest.

REFERENCES

[1] Chao, S.C., Young, D.G., (2000) Screening for inhibitory activity of essential oils on selected bacreria, fungi and viruses. Journal of Essential Oil Research 12: 630-649.

[2] Schwämmle, B., Winkelhausen, E., Kuzmanova, S., Steiner, W., (2001) Isolation of carvacrol assimilating microorganisms. Food Technology and Biotechnology 39: 341-345.

[3] Howell, C.R., Beier, R.C. and Stipanuvic, R.D. (1988) Production of Ammonia by *Enterobacter cloacae* and it's possible role in biological control of *Pythium* preemergence Damping-off by the bacterium. Phytopathology, 78: 1075-1078.

[4] Schütz, A., Golbik, R., Tittmann, K., Svergun, D.I., Koch, M.H.J., Hübner, G. and König, S. (2003) Studies on structure–function relationships of indolepyruvate decarboxylase from *Enterobacter cloacae*, a key enzyme of the indole acetic acid pathway. European Journal of Biochemistry 270: 2322–2331.

[5] Bassler, B.L, Yu, C., Lee, Y.C., Roseman, S. (1991) Chitin-utilization by marine bacteria: degradation and catabolism of chitin-oligosaccharides by *Vibrio furnissi*. Journal of Biological Chemistry 266: 24276–24286.

[6] Watanabe, T., Oyanagi, W., Suzuki, K., Tanaka, H. (1990) Chitinase system of *Bacillus circulans* WL-12 and importance of chitinase A1 in chitin degradation. Journal of Bacteriology 172: 4017–4022.

[7] Watanabe, T., Kimura, K., Sumiya, T., Nikaidou, N., Suzuki, K., Suzuki M, Taiyoji M, Ferrer S, Regue, M. (1997) Genetic analysis of the chitinase system of *Serratia marcescens* 2170. J Bacteriol 179:7111–7117.

[8] Baysal, Ö., Lai, D., Xu, H-H., Siragusa, M., Çalışkan, M., Carimi, F., Teixeria da Silva, J.A., Tör, M. (2013) A proteomic approach provides new insights into the control of soil-borne plant pathogens by Bacillus species. PLoS ONE 8(1): e53182. 1-12.

[9] Müller-Riebau, F., Berger, B., Yeğen, O. (1995) Chemical Composition and Fungitoxic Properties to Phytopathogenic Fungi of Essential Oils of Selected Aromatic Plants Growing Wild in Turkey. Journal of Agricultural and Food Chemistry 43:2262-2266

[10] Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., Boskou, D. (1993) Composition and antioxidant activity of essential oils from Oregano plants grown wild in Greece. Zeitschrift für Lebensmittel Untersuchung und Forschung 197: 20-23.

[11] Deighton, N., Glidewell, S.M., Deans, S.G., Goodman, B.A. (1993) Identification by epr spectroscopy of carvacrol and thymol as the major sources of free radicals in the oxidation of plant essential oils. Journal of Science Food Agriculture 63: 221–225.

[12] Chamberlain, E.M., Dagley, S. (1968) The metabolism of thymol by a *Pseudomonas*. Biochemistry Journal 110:755–762.

[13] King E. O., Ward M. K. and Raney D. E. (1954) J. Lab and Clin Med 44:301-307.

[14] Baysal, Ö. (1997) Determination of micro-organisms decomposing essential oils of *Thymbra spicata* L. var *spicata* and effect of these micro-organisms on some soil-borne pathogens. M.S. Thesis, Akdeniz University, Antalya (in Turkish).

[15] Silme, R.S. (2004) The investigation of "AkseBio", the product developed as a plant activator, for the effect mechanism to some fungal soilborne plant pathogens. M.S. Thesis, Akdeniz University, Antalya (in Turkish).

[16] Yeğen, O., Silme, R.S., Zeller, W. (2004) Der Wirkungsmechanismus von AkseBio-2 und BioZell2000-B als Pflanzenstärkungsmittel. Hamburg Universität, 20 - 23 September 2004. 54.Deutsche Pflanzenschutztagung (54th German Plant Protection Conference) 457.

[17] Silme, R.S. and Yegen, O. (2006) *Thymbra spicata* L. var. *spicata* essential oil assimilation by a selected by a selected bacterium and its affect to control of soil-

borne pathogens. In: I International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation. Acta Horticulturae (ISHS) 723:471-476.
[18] Torres-Rubio, M.G., Valencia-Plata, S.A., Bernal-Castillo, J., Martinez-Nieto, P. (2000) Isolation of *Enterobacteria*, *Azotobacter* sp. and *Pseudomonas* sp., producers of Indole-3-Acetic Acid and siderophores from Colombian rice rhizosphere. Revista Latinoamericana de Microbiologia 42:171-176.
[19] Duncan, D. B. (1955) Multiple range and multiple F tests. Biometrics 11:1-42.
[20] Burkhead, K.D., Schisler, D.A., Slininger, P.J. (1995) Bioautography shows antibiotic production by soil bacterial isolates antagonistic to fungal dry rot of potatoes. Soil Biology & Biochemistry 27: 1611-1616.
[21] Isaacson, P.J., Webster, J.M. (2002) Antimicrobial activity of *Xenorhabdus* sp. R10 (*Enterobacteriaceae*) symbiont of the entomopathogenic nematode, *Steinernema riobrave* (Rhabditida: Steinernematidae). Journal of Invertebrate Pathology 79: 146-153.
[22] Trudel, J., Asselin, A. (1989) Detection of chitinase activity after polyacrylamide gel electrophoresis. Anal. Biochem. 178: 362-366.
[23] Wilson, C.M. (1983) Staining of proteins on gels: comparisons of dyes and procedures. Methods Enzymol. 91: 236-247.
[24] Formácek, V., Kubeczka, K.H. (1982) Essential oil analysis by capillary gas chromatography and carbon-13 NMR spectroscopy, John Wiley Sons, Chichester.
[25] Savithiry, N., Gage, D., Fu, W., Oriel, P. (1998) Degradation of Pinene by *Bacillus pallidus* BR425. Biodegradation 9: 337-341.
[26] Heyen, U., Harder, J. (2000) Geranic acid formation, an initial reaction of anaerobic monoterpene metabolism in denitrifying *Alcaligenes defragrans*. Applied and Environmental Microbiology 66: 3004-3009.
[27] Grimont, F. and Grimont, P.A.D. (1992) The genus *Enterobacter*. In: A. Balows., H.G. Triiper and M. Dworkin (ed), The Prokaryotes Vol. III. 2nd edn. Springer-Verlag, New York, pp 2797–2815.
[28] Vančura, V. and Hanzlikova, N. (1972) Root Exudates of Plants IV. Differences in Chemical Composition of Seed and Seedlings Exudates. Plant and Soil 36: 271-282.
[29] McInroy, J.A. and Kloepper, J.W. (1995) Survey of Indigenous Bacterial Endophytes from Cotton and Sweet Corn. Plant and Soil 173: 337-342.
[30] Morales-García, Y.E., Juárez-Hernández, D.A., Aragón-Hernández, C., Mascarua-Esparza, M.A., Busti- llos-Cristales, M.R., Fuentes-Ramírez, L.E., Martínez-Contreras, R.D. and Muñoz-Rojas, J. (2011) Growth Response of Maize Plantlets Inoculated with *Enterobacter* sp., as a Model for Alternative Agriculture. Revista Argentina de Microbiología 43: 287-293.
[31] Zakria, M., Ohsako, A., Saeki, Y., Yamamoto, A. and Akao, S. (2008) Colonization and Growth Promotion Characteristics of *Enterobacter* sp. and *Herbaspirillum* sp. on *Brassica oleracea.* Soil Science and Plant Nutrition 54: 507-516.
[32] Al-Mughrabi, K.I. (2010) Biological control of *Fusarium* dry rot and other potato tuber diseases using *Pseudomonas fluorescens* and *Enterobacter cloacae*. Biological Control 53: 280-284.
[33] Kang, S.H., Cho, H.S., Cheong, H., Ryu, C.M., Kim, J.F. and Park, S.H. (2007) Two bacterial endophytes eliciting boot plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). Journal of Microbiology and Biotechnology 17: 96-103.
[34] Hardoim, P.R., Van Overbeek, L.S. and Van Elsas, J.D. (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology 16: 463-471.
[35] Zhao, Y. (2010) Auxin Biosynthesis and Its Role in Plant Development. Annual Review of Plant Biology, 61: 49-64.
[36] Frankenberger, W.T., Jr, Brunner, W. (1983) Methods of detection of auxin-indole acetic acid in soil by high performance liquid chromatography. Journal of American Soil Society 47: 237-241.
[37] Lynch, J.M. (1985) Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In: Soil Organic Matter and Biological Activity. Vaughan D and Malcom RE (ed) Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, Boston, Lancaster, pp 151-174.
[38] Schütz, A., Sandalova, T., Ricagno, S., Hübner, G., König, S. and Schneider, G. (2003) Crystal structure of thiamindiphosphate-dependent indolepyruvate decarboxylase from *Enterobacter cloacae*, an enzyme involved in the biosynthesis of the plant hormone indole-3-acetic acid. European Journal Biochemistry 270: 2312-2321.
[39] Yue, Q., Miller, C.J., White, J.F., Jr, Richardson, M.D. (2000) Isolation and characterization of fungal inhibitors from *Epichloe festucae*. Journal of Agricultural Food Chemistry 48: 4687-4692.
[40] Pal, K.K., Tilak, K.V., Saxena, A.K., Dey, R., Singh, C. (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth

promoting rhizobacteria. Microbiological Research 156: 209-223.
[41] Dileep, K.V., Tintu, I., Remya, C., Haridas, M. and Sadasivan, C. (2012) Studies of IAA and IBA as fungal α-amylase inhibitors using enzyme kinetics, molecular modeling and thermodynamics. Starch/Stärke 64: 991–995.
[42] Choma, I.M., Grzelak, E.M. (2011) Bioautography detection in thin-layer chromatography. Journal of Chromatography A 1218: 2684-91.
[43] Homans, A.L., Fuchs, A. (1970) Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. Journal of Chromatography A 51: 327-329.
[44] Guleriaa, S., Kumarb, A. and Tikua, A.K. (2008) Chemical composition and fungitoxic activity of essential oil of *Thuja orientalis* L. grown in the north-western Himalaya. Z Naturforsch 63: 211-214.
[45] White, G.F., Snape, J.R. and Nicklin, S. (1996) Biodegradation of Glycerol Trinitrate and Pentaerythritol Tetranitrate by *Agrobacterium radiobacter*. Applied Environmental Microbiology 62: 637-642.
[46] Yazdani, S.S, Gonzalez, R. (2008) Engineering *Escherichia coli* for the efficient conversion of glycerol to ethanol and co-products. Metabolic Engineering 10: 340–351.
[47] Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R.H., (Eds.), 8th Ed. (2003) Manual of Clinical Microbiology, ASM, Washington, D.C.
[48] Misenheimer, T. J., Anderson, R. F., Lagoda, A. A., Tyler, D. D. (1965) Production of 2-ketogluconic acid by *Serratia marcescens*. Appl. Microbiol. 13: 393-396.
[49] Shinagawa, E., Matsushita, K., Adachi, 0., Ameyama, M. (1978) Membrane-bound D-gluconate dehydrogenase of *Serratia marcescens:* purification and properties. Agric. Biol. Chem. 42: 2355-2361.
[50] Neijssel, O. M., Tempest, D. W., Postma, P. W., Duine, J. A., Frank Jzn J. (1983) Glucose metabolism by K'-limited *Klebsiellu aerogenes*: evidence for the involvement of a quinoprotein glucose dehydrogenase. FEMS Microbiol. Lett. 20 :35-39.
[51] Bouvet, 0.M.M., and Grimont, P.A.D. (1987) Diversity of phosphoenolpyruvatelglucose phosphotransferase system in the Enterobacteriaceae. Ann. Inst. Pasteur (Paris) 138:3-13.
[52] Bascomb, S., Lapage, S. P., Curtis, M. A., Wilcox, W. R. (1973) Identification of bacteria by computer: identification of reference strains. J. Gen. Microbiol. 72291-315.
[53] Grimont, P. A. D., Grimont, F., Dulong de Rosnay, H. L. C., Sneath, P. H. A., (1977) Taxonomy of the genus *Serratia*. J. Gen. Microbiol. 98: 39-66.
[54] Katznelson, H., Tanenbaum, S. W., Tatum, E. L. (1953) Glucose, gluconate and 2-ketogluconate oxidation by *Acetobacter melanogenum*. J. Biol. Chem. 204: 43-59.
[55] Bernaerts, M., and De Ley, J. (1971) 2,5-Diketogluconate formation by *Chromobacterium*. Antonie van Leeuwenhoek J. Microbiol. Serol. 37: 185-195.
[56] Gossele, F., Swings, J., De Ley, J. (1980) A rapid, simple, and simultaneous detection of 2-keto-, 5-keto- and 2,5-diketogiuconic acids by thin layer chromatography in culture media of acetic acid bacteria. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 1:17-181.
[57] Gilbert, E.S. and Crowley, D.E. (1997) Plant compounds that induce polychlorinated biphenyl biodegradation by *Arthrobacter* sp. strain B1B. Appl. Environ. Microbiol. 63, 1933-1938.
[58] Hernandez, B.S., Koh S.C., Chial M., Focht D.D. (1997) Terpene-utilizing isolates and their relevance to enhanced biotransformation of polychlorinated biphenyls in soil. Biodegradation 8, 153–158.
[59] Chadwick, A.V. and Burg, S.P. (1970) Regulation of root growth by auxin-ethylene interaction. Plant Physiology 45:192-200.
[60] Gehring, C.A., Irving, H.R. and Parish, R.W. (1990) Effects of auxin and abscisic acid on cytosolic calcium and pH in plant cells. Proceeding of National Academic Science 87: 9645-9649.
[61] Russell, J.B. and Dombrowski, D.B. (1980) Effect of pH on the Efficiency of Growth by Pure Cultures of Rumen Bacteria in Continuous Culture. Applied Environmental Microbiology 39: 604-610.
[62] Garland, P.B. (1977) Energy transduction in microbial systems. Symp Soc Gen Microbiol 27: 1-21.
[63] Riebeling, V., Thauer, K.K. and Jungermann, K. (1975) The internal-alkaline pH gradient, sensitive to uncoupler and ATPase inhibitor, in growing *Clostridum pasteurianum*. European Journal of Biochemistry 55: 445-453.
[64] Bonkowski, M., Brandt, F. (2002) Do soil protozoa enhance plant growth by hormonal effects? Soil Biology & Biochemistry 34: 1709-1715.
[65] Marschner, P., Crowley, D., Rengel, Z. (2011) Rhizosphere interaction between microorganisms and plants govern iron and phosphorus acquisition along the root axis -

model and research methods. Soil Biology and Biochemistry 43: 883-894.
[66] Baysal, Ö. and Tör, M. (2014) Smart Biologics For Crop Protection in Agricultural Systems. Turkish Journal of Agriculture and Forestry. 1-29.

Received: **14.10.2015**
Accepted: **28.01.2016**

CORRESPONDING AUTHOR

Omur Baysal
Muğla Sıtkı Koçman University
Faculty of Science
Department of Molecular Biology and Genetics
48000 Muğla – TURKEY

e-mail: omurbaysal@mu.edu.tr