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EFFECT OF SOME PLANT GROWTH PROMOTING AND BIOAGENT BACTERIA ON DEGRADATION OF ORGANOCHLORINE PESTICIDES

Kenan Karagoz [1,3], Fatih Dadasoglu [1,3], Recep Kotan [2]

[1]Agri Ibrahim Cecen University, Faculty of Science and Literature, Department of Molecular Biology and Genetics 04100, Agri, Turkey
[2]Ataturk University, Agricultural Faculty, Department of Plant Protection 25240 Erzurum, Turkey
[3]Agri Ibrahim Cecen University, Central Research and Application Laboratory, 04100, Agri, Turkey

ABSTRACT

In this study, we aimed to define the biodegradation ability of some bacteria which have been used in different studies as plant growth promoting bacteria (PGPB) or bioagent, on some organochlorine pesticides. Five bacterial strains (*Bacillus megaterium* KBA-10, *Pantoea agglomerans*, RK-79, RK-92, RK-169 and RK-198) have positive effects on plant growth parameters and have suppressive effect on some plant diseases. The bacterial strains also showed degradation effect on α-HCH, Hexaclorobenzene, γ-HCH, Heptaclor, Aldrin, α-Endosulfan, Dieldrin, and o'p DDD in-vitro. All strains degraded the organochlorine pesticides in different values. Degradation efficiency assessed for Alfa-HCH ranged from 67.8 % to 90.3 %; for Hexaclorobenzene from 66.3 % to 90.1 %, for γ-HCH from 67.5 % to 90.4 %, for Heptaclor from 73.8 % to 90.4 %, for Aldrin from 73.7 % to 92.0 %, for Alfa-Endosulfan from 66.1 % to 87.7 %, for Dieldrin from 67.9 % to 89.7, for op DDD from 51.4% to 88.7% in-vitro. Our data showed that all stains have ability of degradation of the organochlorine pesticides and it is expected that different microbial fertilizers derived from this strains could promote degradation of the organochlorine pesticides.

KEYWORDS:
Organochlorine pesticides, PGPB, biodegradation, MIS, BIOLOG

INTRODUCTION

Application of pesticides is a worldwide problem. Because of toxicity and residue, pesticides have adverse effect on humans and environment. In addition, pesticide usage was considered an agricultural stress reason [1]. Organochlorine pesticides (POPs) are persistent pollutants which were first introduced in the 1940s and used extensively until 1960s in agriculture. Some representative compounds in this group are DDT, dieldrin, chlordane, mirex, kepone, lindane, and benzene hexachloride. People can be exposed to organochlorine pesticides through accidental inhalation if they are in an area where POPs were recently applied. The chemicals can also be ingested in fish, dairy products, and other fatty foods that are contaminated. Exposure to organochlorine pesticides over a short period may produce convulsions, headache, dizziness, nausea, vomiting, tremors, confusion, muscle weakness, slurred speech, salivation and sweating. Long-term exposure to organochlorine pesticides may damage the liver, kidney, central nervous system, thyroid and bladder. Many of these pesticides have been linked to elevated rates of liver or kidney cancer in animals. There is some evidence indicating that organochlorine pesticides may also cause cancer in humans.

The organochlorine pesticides accumulate in environment because they are not completely biodegradable, or are degraded very slowly [2] As damage of POPs came out later, use of some POPs were banned in 1970s and 1980s. Despite POPs were banned in some countries, residues of this group pesticides can linger in environment [3]. Furthermore, some POPs like endosulfane and lindane (γ-HCH) are still used worldwide. So eliminating of POPs is an important issue [4]. One of the approaches is use of the microorganisms to degrade pesticides.

Microorganisms are biogeochemical agents which play a key role on environment. They convert organic components to simple inorganic components. Like most pollutants, pesticides are also degraded by microorganism [5] Degradation of pesticides microbiologically was considered as effective and low cost process[6] so there are numerous studies on degradation of organochlorine pesticides by microorganisms, especially bacteria[7-11]

PGPBs have positive effect on growth of plants. The plant growth effect of PGPBs have been explained with mechanisms such as production of phytohormones, nitrogen fixation, solubilization of

phosphate and suppression of pathogens [12] In recent years, PGPB's are often researched in a great number of plants. Some of these plants are rice [13], barley [14], sugar beet [15], lettuce [16, 17], strawberries [18], peanut [19], sour cherry [20], apple [21], and plum [22]. As shown, PGPB's were used in a wide range of plants and researchers observed positive effects on plant growth parameters and suppression of some diseases with single or mixed inoculation of PGPBs in different value.

The aim of this study is to determine biodegradation effects of five bacterial strains, which are studied in different plants and conditions [17, 20, 22, 23] as PGPB and biocontrol agent, on some organochlorine pesticides.

MATERIALS AND METHODS

Bacterial strains and chemicals. The five bacterial strains, *Bacillus megaterium* KBA-10, *Pantoea agglomerans* RK-79, RK-92, RK-169, and RK-198, used in this study were previously isolated from different sources. All of these strains have nitrogen fixation and phosphate solubilizing ability to various extents. α-HCH, Hexaclorobenzene, γ-HCH, Heptaclor, Aldrin, α-Endosulfan, Dieldrin and o'p DDD were purchased from Dr. Erenstrofer, Ausbury, Germany

Hypersensitivity tests (HR). All of the bacterial strains were tested for hypersensitivity on tobacco plants (*Nicotiana tabacum* L. var. Samsun). The bacterial suspension (10^8 cfu /ml) was prepared in sterile distilled water and infiltrated into the inter-costal area of the leaves of tobacco plants by using a 3-cc syringe (Becton Dickinson, Franklin Lakes, NJ, USA). The inoculated plants were incubated in a completely randomized design on the greenhouse bench for 24–48 h at 25–28 °C. The presence of rapid tissue necrosis at the inoculation site was recorded within 24–48 h after infiltration. This test was repeated, three times for each strain. Sterilized distilled water (sdH2O) was used as control.

Identification of the bacterial strains by microbial identification system (MIS). Identification of the tested bacterial strains was confirmed by using MIS systems. Preparation and analysis of FAMEs from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer's manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMEs were separated by gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm x 0.25 μm with cross linked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 50) with the MIS software package

Identification of the bacterial strains by Biolog micro plate system (BIOLOG). Identification of the tested bacterial strains was confirmed by using BIOLOG systems. One day before the inoculation of Biolog GN2 and GP2 plates, bacterial strains were streaked on TSA or BUG agar plates. Each well of Biolog GN2 or GP2 micro-titer plates was inoculated with 125 μl of the Gram-negative or positive bacterial suspension, respectively, adjusted to the appropriate density (10^8 cfu/ml) and incubated at 26 ± 2 °C for 24 and 48 h. The development of color was automatically recorded using a micro plate reader with a 590-nm wavelength filter. Identification (Biolog Microlog 34.20 database) and ASCII file output of test results, applying the automatic threshold option, were performed using BIOLOG420/ Databases/ GN601 and GP601 KID software [24]. Carbon source utilization rates of the strains were estimated as percentages.

Prepare inoculum and pesticides for determination of degradation. The experiments were monitored according to [8] with minor modification. For this purpose, pesticide mix was prepared including α-HCH, Hexaclorobenzene, γ-HCH, Heptaclor, Aldrin, α-Endosulfan, Dieldrin and o'p DDD at same concentration. Then 100 ml Erlenmeyer flasks with medium (TSB) were autoclaved at 121ºC for 20 min. These flasks were spiked with POPs to a concentration of 200 mg l^{-1}. The flasks were inoculated with 1ml bacterial suspension adjusted to a set optical density of (OD_{600} = 1). These inoculated flasks were incubated at 26±2 ºC on an orbital shaker at 150 rpm for 7 days. Uninoculated flasks (control) were also prepared to check the abiotic degradation under the same conditions. This procedure was carried out in triplicate and results are means of three measurements.

TABLE 1
MIS and BIOLOG identification results of bacterial strains, their similarity index (SI), hypersensitivity test (HR) results and carbon source utilization rates (CSU, %)

Strain number	MIS identification result	SIM%	Biolog identification result	SIM%	HR	CSU %	Isolated From
KBA-10	Bacillus megaterium	49	Bacillus amyloliquefaciens	16	-	36,84	apricot (root)
RK-79	Pantoea agglomerans	76	Pantoea agglomerans	55	-	63,15	apple (shoot)
RK-92	Pantoea agglomerans	88	Pantoea agglomerans	58	-	53,68	pear (shoot)
RK-169	Pantoea agglomerans	78	Pantoea agglomerans	35	-	52,63	apple (shoot)
RK-198	Pantoea agglomerans	77	Pantoea agglomerans	79	-	63,15	Pear (shoot)

-: HR test is negative in tobacco

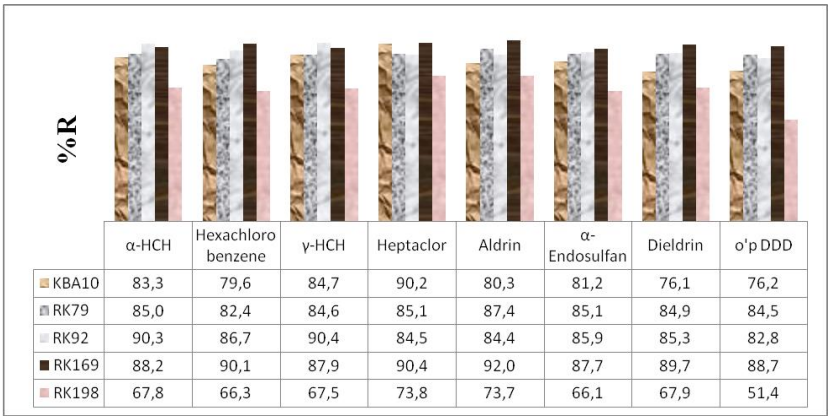
Assessment of Pesticide biodegradation. The experimental and control samples were extracted with dichloromethane. The extracts were dehydrated by passing them through anhydrous Na_2SO_4 and concentrated using a rotary evaporator. The residue was re-dissolved in 5ml of acetonitrile and 2 ml were injected into the GC/MS. The extract was analyzed with a gas chromatograph (Shimadzu GC-2010) equipped with mass spectrometer (GCMS- QP2010), auto injector (AOC-20i) and a DB-5MS capillary column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 um), coupled to MS via direct interface. The injector and detector were operated at 250 and 280 ºC respectively. The initial oven temperature was 90 ºC and set for a linear increase of 5 ºC min^{-1} to a final temperature 300 ºC. The helium was used as carrier gas at a flow rate of 1.85 ml min^{-1}. The ion source temperature was 200 ºC while interface temperature was 270 ºC. The electron impact (EI) mass spectra were obtained at 70 eV and monitored in the range of 50 to 400 m/z [8]. GC/MS data of pesticides were given in Table 2. Finally, the percentage reduction of pesticide value was determined according to the following equation [25].

%R =100 - [(Hpesticide sample/Hpesticide control) X 100]

RESULTS

Bacterial strains, identification and test results. MIS and BIOLOG identification result and some characteristics of bacterial strains were given in Table1. All isolates have negative test result on tobacco. Identification results of strain KBA 10 are different between MIS (as *Bacillus megaterium*) and BIOLOG (as *Bacillus amyloliqefaciens*) in species level but same in genus level. Other isolates were identified as *Pantoea agglomerans* by MIS and BIOLOG. Carbon sources utilization percentages of isolates on GN2 and GP2 plates range from 38.84% to 63.15%.

FIGURE 1
Biodegradation efficiency (R %) of bacterial strains



	α-HCH	Hexachloro benzene	γ-HCH	Heptaclor	Aldrin	α-Endosulfan	Dieldrin	o'p DDD
KBA10	83,3	79,6	84,7	90,2	80,3	81,2	76,1	76,2
RK79	85,0	82,4	84,6	85,1	87,4	85,1	84,9	84,5
RK92	90,3	86,7	90,4	84,5	84,4	85,9	85,3	82,8
RK169	88,2	90,1	87,9	90,4	92,0	87,7	89,7	88,7
RK198	67,8	66,3	67,5	73,8	73,7	66,1	67,9	51,4

TABLE 2
GC/MS data

Pesticide	RT (min)	Parent ion (m/z)	Reference ion
α-HCH	16,14	183	219
Hexaclorobenzene	16,50	284	142
γ-HCH	17,29	183	219
Heptaclor	19,80	100	272
Aldrin	20,96	66	79
α-Endosulfan	23,28	195	214
Dieldrin	24,04	79	108
o'p DDD	24,93	235	165

RT: retention time

Biodegradation efficiency of bacterial strains. According to our result all of five bacterial strains are capable of degrading the organochlorine pesticides in different values, the result were given in Figure 1. Most effective strains were determined as RK-92 for degradation of α-HCH and γ-HCH with efficiency 90,3 % and 90,4 % respectively, and determined as RK-169 for degradation of Hexaclorobenzene, Heptaclor, Aldrin, α-Endosulfan, Dieldrin and o'p DDD with efficiency 90,1 %, 90,4 %, 92 %, 87,7 %, 89,7 % and 88,7 % respectively. It was detected that other strains (KBA-10 and RK-79) have also high value degradation activity. For degradation of all pesticides, *Pantoea agglomerans* RK-198 was defined as least effective strain.

DISCUSSION AND CONCLUSION

When data were evaluated, similar components (α-HCH - γ-HCH) were degraded by RK-92 and the others were depredated by RK-169 at the highest level. In this study, any analysis were not performed for defining the degradation enzymes of bacteria but it is known that degradation of some components depend on existence of appropriate degradation enzymes beside of environmental conditions [26]. So RK-92 and RK-169 may produce different enzymes. Like can be seen above RK-198 has not powerful degradation activity, as much as the other *Pantoea agglomerans* strains. As stated above, strains may have different enzyme production ability and biodegradation activity probably is a strain dependent facilities.

Effects of the strains used in this study as PGPB or bioagent were researched in different conditions. Strain KBA10 was used in lettuce as PGPB and bioagent against to bacterial leaf spot of lettuce. It was determined that KBA10 have positive effect on plant growth parameters and pretty much inhibit occurrence of disease. In the same study; it was screened that strain RK-169 and RK-198 inhibited the disease[17] Strain RK-92 was tested in apricot, apple, sugar beet, bean and it is considered very useful as biofertilizer. RK-79 was also used in plum and apricot. Positive effects of this strain on shoot length or diameter and fruit set were determined [22, 23].

Another beneficial effect of the five bacterial strains was screened with this study. According to result all strains have ability degradation of the POPs in different values. In literature, there are so many records about POPs degradation by bacteria. It was determined that *Streptomyces* sp., [27], *Xanthomonas* sp., [28], *Mycobacterium* sp. [29] can degrade lindane with 81%, 100% and 96% efficiency, respectively [30]. Endosulfan degradation abilities of different bacterial strains were reported in another study with 40% - 93% efficiency [8]. Our results were similar with literature data. Strains used in this study degrade different POPs in value 51.0% - 92.0%.

As mentioned before, there are so many strains which have ability of degradation of POPs were reported. In addition to, it is known that *Bacillus* strains can degrade DDT and γ-HCH [31] and *Pantoea agglomerans* strains also have ability degradation of DDT and γ-HCH [32]. Beside of the stains used in this study can degrade eight different POPs at different rate, the strains have some abilities like high efficiency nitrogen fixation and phosphate solubilization or provide resistance to plant disease using different mechanisms [17].

When considering all of above, it is thought that microbial fertilizers and biopesticides derived from these strains might be convenient in sustainable agriculture. As known, pesticide usage is a huge worldwide problem and pesticides are mostly permanent in environment. Many researcher works on solutions for reducing pesticide usage or eliminating pesticide residues from environment. We believe that applications of beneficial microorganisms may serve as useful instruments for both reducing pesticide usage and degradation of pesticide residues in environment.

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CORRESPONDING AUTOR

Kenan Karagoz,
Agri Ibrahim Cecen University, Faculty of Science and Literature, Department of Molecular Biology and Genetics 04100, Agri, TURKEY

Email: kkaragozagri@gmail.com