Isolation and Identification of Actara 25 WG Degrading Bacteria from Agricultural Soils and Evaluation of Their Activity

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Abstract

Fifty five agricultural soil samples were collected from different sites in Hilla city. These samples were screened for Actara 25 WG (Pesticide) degrading bacteria. Pour plate method involving the use of serial dilutions was employed for the isolation of bacteria. The isolated bacterial species were identified on the basis of morphological and biochemical characteristics. The results explained that these isolates belonged to three different species including *Pseudomonas aeruginose Aeromonas hydrophila* and *Psendomonas putida*. The activity of these isolates for pesticide degradation was evaluated due to increasing of viable count (CFU/ ml) , decreasing of pH value, and increasing of optical density ($0.D_{600}$). The results revealed all of these isolates have an ability of biogegradation , but the more active one is *P. putida* which increase the viable count to 89×10^8(CFU/ ml) and the $0.D_{600}$ to (1.7) during the incubation period (7 days). So the present study demonstrated that the isolated bacterial species could be harnessed to use in bioremediation of agricultural lands polluted with pesticide .

Key words: Actara 25 WG , Pesticide , agricultural soil

الخلاصة

تم جمع 55 عينة تربة زراعية من مواقع مختلفة في مدينة الحلة. وقد اختبرت قابلية البكتريا المعزولة من هذه الترب على التكسير الحيوي للمبيد الحشري Actara 25 WG وقد استخدمت طريقة الصب بالاطباق باستعمال التخافيف العشرية لغرض عزل البكتريا. وقد شخصت الانواع البكتيرية اعتماداً على الصفات المظهرية والكيموحيوية. وقد اظهرت النتائج ان العزلات تعود الى ثلاث انواع مختلفة هي *P. aeruginosa*, *A. hydrophila* , *P.putida* . كما اختبرت كفاءة هذه العزلات في التكسير الحيوي للمبيد المستعمل بدلالة الزيادة في عدد الخلايا الحية / مل للمزروع البكتيري والانخفاض في قيم دالة الحموضة وكذلك الزيادة بالكثافة الضوئية للمزروع خلال فترة الحضانة (7 ايام). وقد اظهرت جميع العزلات قابلية التكسير الحيوي للمبيد الحشري المستعمل الا ان العزلة الاكثر كفاءة كانت *P. putida* والتي زادت العدد البكتيري الحي الى 89×10^8 وكذلك ادت الى زيادة الكثافة الضوئية الى 1.7 خلال فترة الحضانة. لذلك توضح الدراسة الحالية امكانية تسخير العزلات البكتيرية في التردي الحيوي للمبيدات الملوثة للترب الزراعية.

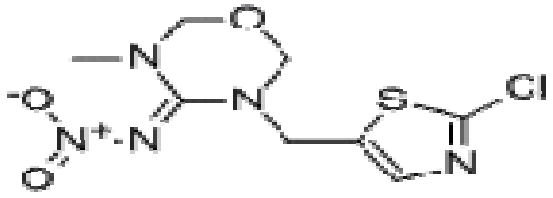
الكلمات المفتاحية : المبيد الحشري . البكتريا المحللة للمبيدات . الترب الزراعية

Introduction

Insecticides still remain a very important component among the strategies for effective control of mirids and other major insect. Pests of different agricultural products in Iraq, in general ,pesticides are poisonous chemicals or kill non-target plants and animals or human [Ecobichon, 2001].These compounds (insecticides) have a pivoted role in our lives,not only for crop protection in agriculture activities, but also to avoid the spreading of harmful pests causing human disease such as Malaria [Herne *et al*., 2002].

The insecticides used in the present investigation was Actara 25WG.This compound is :3-{(2-chloro -5-thiazolyl)methyl} tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxidiazin-4-imine.It has as its component the major active ingredient, tiamethaxam 25%

[Bhagobaty *et al*., 2007].Its mode of action was with contact, stomach and systematic activity [Anikwe *et al*., 2009].It has harmful effect on human when it cause cytotoxicity cell death, both as single cell necrosis and increased cell replication rates. And empirical formula is $C_8H_{10}C_1N_5O_3S$,whil its structural formula is givin below:-



Its belong to the Neoicotinoide group, and it has as its component the major active ingredient.

Pesticides reaching the soil in significant quantities have direct effect on soil microbiological aspects, which in turn influence plant growth. Some of the most important effects caused by pesticides are: (1) alteration in ecological balance of soil microflora., (2) continued application of large quantities of pesticide may cause ever lasting changes in the soil microflora (3) adverse effect on soil fertility and crop productivity, (4) inhibition of N_2 fixation in soil, (5) suppression of nitrifying *Nitrosomonas* and *Nitrobacter* by soil fumigants ethylene bromide, telone and vapam have also been reported, (6) alteration in nitrogen balance of the soil, (7) interference with ammonification in soil , (8) adverse effect on mycorrhizal sysmbioses in plants and nodulation in legumevs and (9) alterations in the rhizosphere microflora both quantitatively and qualitatively[Maria *et al*., 2002].

The present study aimed to evaluate the potential activity of isolated bacterial strains from contaminated agriculture soils to Actara25WG biodegradation.

Material &Method

The 50 soil sample were collected from different agriculture sites in Hilla during September to November,2012. A quantity of 1gm of soil sample was suspended in 100 ml of minimal salts medium containing:$CaCl_2$ (0.02gm), $MgSo_4$. $7H_2O$(0.2gm),K_2HPO_4(1gm) ,KH_2PO_4(1gm),$(NH_4)_2SO_2$ (1gm), $Fecl_3$ (0.0001gm).All these salts were dissoloved in one litter of distilled water. Three concentrations of Actara 25WG were used as a sole source of carbon and energy which were (0.1,0.01 and 0.001%) then incubated in 250ml flasks (each one containing 100 ml) at $37c^0$ (Nagamani *etal*.,2009).

A volume of 5ml of enriched media was transferred into freshly prepared which supplemented with the insecticide. The single colonies were streaked onto blood agar plates and incubated at $37c^o$ for 24-48 hr.

Identification of isolates:-

The Bacterial isolates were identified according to the morphological observation and biochemical characterization. The tests involved were gram staining, oxidase tests. catalase, amylase and gelatinase production, Indole, Methylen red, citrate utilization(Macfaddin,2000).

Efficasy of degrading isolated:-

The efficacy of Actara 25WG degrading bacterial stains under laboratory conditions of temperature ($37c^o$) where tested for three concentration of (0.1,0.01and 0.001%).

During the incubation period (7 days) the following parameters were evaluated: pH of culture medium , colony forming unit (CFU/ml) and optical density at 600 nm for broth culture.

Viable Cell Count Determination

Aliquots (2.5ml) of 24hr old bacterial cultures grown in mineral salts medium(MSM) were inoculated into 100ml Elenmeyer flasks containing 25ml of nutreint broth supplemented with various concentrations (0.1 , 0.01 , 0.001%) of Actara 25 WG to test their ability to degrade the supplemental substrate (pesticide). A control was maintained with equal volume of broth containing bacterial culture , but without pesticide. Bacterial growth was followed by viable cell counts immediately after inocnlum at 1, 2, 3, 4, 5, 6, 7 days of incubation . A bacterial inoculum (1ml) was drawn at regular intervals from the test control cultures and serial dilutions were performed using (9) ml of sterile saline (0.85% of NaCl; pH 7). Appropriate dilutions were plated in triplicate on nutrient agar and the plates incubated at 37C^{o} for 24 hr. The bacterial colonies were counted (CFU/ml) with a colony counter (Mallick *et al*., 1999).

Result and Discussion

- Bacterial isolation and identification:-

Agriculture soils contaminated with insecticides was chosen as the source of degrading microorganisms isolated in this study.

soils sample were enriched in sterile MSM medium using Actara 25WG (insecticides) as a sole source for carbon and energy. The samples were further treated with Actara to ensure that only degrading isolated which have an ability to grown in the presence of this toxic compounds, would be selected.

After treatment only three bacterial strains were grown. These isolates were identified according to the biochemical test and cultural properties (Table -1). The results showed it belong to *Pseudomonas aerugenosa* ,*Aeromonas hydrophilia* and *Pseudomonas putida* according to Bergeys manual of determinative of bacteriology (1994).

Three parameters were tested to evaluate the most active isolates (pH measurement of cultural media, optical density (at 600nm) and CFU/ml during incubation period (7 days).The results revealed the degradation based on high resistance to this xenobiotic compound (Tables 2 to 4).

The degradation performance of selected strains was examined of different Actara 25WG concentrations (0.1,0.01,0.001%). Serial exposure to increasing levels of compound concentrations was used to determine the resistance of isolated strains .

Acclimatization of the microorganisms over comes the substrate inhibition problems that occurred in xenobiotic biodegradation at high concentration (Yang *et al*., 2006).

The growth of bacteria and Actara concentration in media showed the inverse proportion with each other. The decrease in concentration accompanied with increase in biomass (Figurs 1 to 3).

A few species of bacteria such as pseudomonas have been reported able to utilize one or more of pesticides (Paunescu et al., 2009). The present data showed that the diverse group of bacteria isolated from agricultural soils polluted with pesticides have the capacity to grow in the presence of insecticide (Actara 25 WG) as

a sole sourece of carbon and energy. The growth cureves of the three isolates cultured in MSM liquied media amended with insectide but the better one was *P. putida* when increased the viable count to 89×108 CFU/ml concomitant with increasing of optical density to 1.7 nm at the same time decreased pH value to 6.0 at the final of incubation period (Figure 1-3).

These results give an ability to use these isolates in in removal of pesticides from polluted soils.

Table (1):Biochemical characteristics of Actara 25 WG – degrading strains isolated from agricultural soils

Test	I	II	III
Gram's reaction	-	-	-
Cell shape	Small roods	Short roods	Short roods
Spores	-	-	-
Motility	+	+	-
Indole test	+	-	+
Methyl red	+	-	-
Voges-Proskauer test	-	-	+
Catalase	+	+	+
Citrate utilization	+	+	+
Oxidase	-	+	-
Urease	-	-	+
Nitrite reduction	+	+	+
Casein and starch hydrolysis	-	+	-
Gelatin and Tween- 60 hydrolysis	-	-	-
Esculin hydrolysis	-	-	+
Growth on MacConkey agar	+NLF	+	+LF
Ornithine decaboxylase	-	+LF	-
Phosphatase	+	-	+
Acid from glucose, sucrose	+	+	+
Gas from glucose	-	+	+
Growth (Temperature 15^{o}C-42 ^{o}C)	+	+	+
Growth (NaCl-2-5%)	+	+	+
Growth (pH 8-10.5)	+	+	+
Utilization of glucose and adonitol as carbon source	+	+	+
Utilization of L-glutmic acid, L-phenylalanine, L-valine, L-histidine as nitrogen source	+	+	+

I= *P. aeruginosa*, II= *A. hydrophila*, III= *P. putida*

Table (2) Optical density (at 600 nm) of degrading isolates grown on three concentrations of Actara 25 WG during incubation period .

Actara 25 WG conc. / Time (day)	*P.aeruginosa*			*A . hydrophila*			*P.putida*		
	0.1%	0.01%	0.001%	0.1%	0.01%	0.001%	0.1%	0.01%	0.001%
1	0.56	0.63	0.65	0.42	0.51	0.61	0.68	0.70	8.1
2	0.58	0.65	0.68	0.44	0.55	0.63	0.70	0.72	8.5
3	0.60	0.68	0.71	0.48	0.59	0.66	0.73	0.77	8.3
4	0.63	0.70	0.75	0.49	0.61	0.70	0.77	0.82	8.7

5	0.66	0.73	0.79	0.51	0.63	0.73	0.80	0.83	8.9
6	0.68	0.76	0.81	0.53	0.66	0.75	0.82	0.88	8.9
7	0.69	0.79	0.83	0.55	0.68	0.78	0.85	0.90	8.9

Table (3) Measurements of pH of culture media of degrading isolates grown on three concentrations of Actara 25 WG during incubation period .

Actara 25 WG conc. / Time (day)	*P.aeruginosa*			*A . hydrophila*			*P.putida*		
	0.1%	0.01%	0.001%	0.1%	0.01%	0.001%	0.1%	0.01%	0.001%
1	6.6	6.8	6.4	6.9	6.9	6.7	6.5	6.3	6.1
2	6.3	6.5	6.1	6.7	6.8	6.6	6.1	6.0	6.0
3	6.1	6.3	6.0	6.6	6.7	6.5	5.9	5.9	5.7
4	6.0	6.1	5.8	6.5	6.5	6.4	5.9	5.7	5.3
5	6.0	6.0	5.7	6.3	6.4	6.4	5.8	5.6	5.2
6	6.0	5.8	5.6	6.0	6.3	6.3	5.7	5.5	5.1
7	5.8	5.7	5.5	6.0	6.1	6.2	5.5	5.4	5.0

Table (4) Viable count (CFU / ml) × 10^8 of Actara 25 WG degrading isolates grown on three concentrations during incubation period .

Actara 25 WG conc. / Time (day)	*P.aeruginosa*			*A . hydrophila*			*P.putida*		
	0.1%	0.01%	0.001%	0.1%	0.01%	0.001%	0.1%	0.01%	0.001%
1	14.6	18.1	19.9	13.1	14.1	16.1	18.2	20.1	20.2
2	18.1	20.4	23.1	13.3	15.5	18.2	19.8	22.0	22.1
3	20.2	23.3	25.1	14.4	16.9	19.9	20.3	24.4	23.2
4	22.3	26.1	27.7	15.5	18.1	20.1	22.4	26.6	24.1
5	25.5	27.8	29.1	16.7	19.5	23.1	26.2	28.1	26.1
6	28.1	29.1	30.4	18.7	20.2	25.5	28.2	29.9	27.2
7	30.3	31.3	32.1	19.9	22.1	26.1	30.4	33.1	34.1

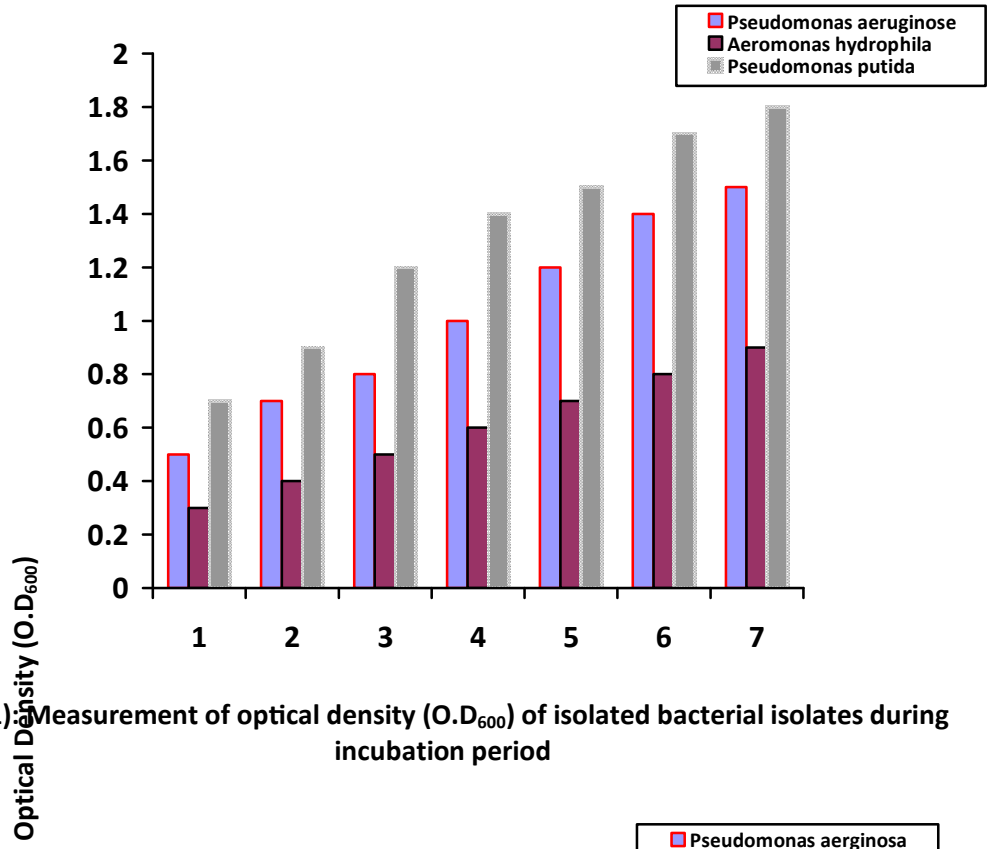


Fig.(1): Measurement of optical density ($O.D_{600}$) of isolated bacterial isolates during incubation period

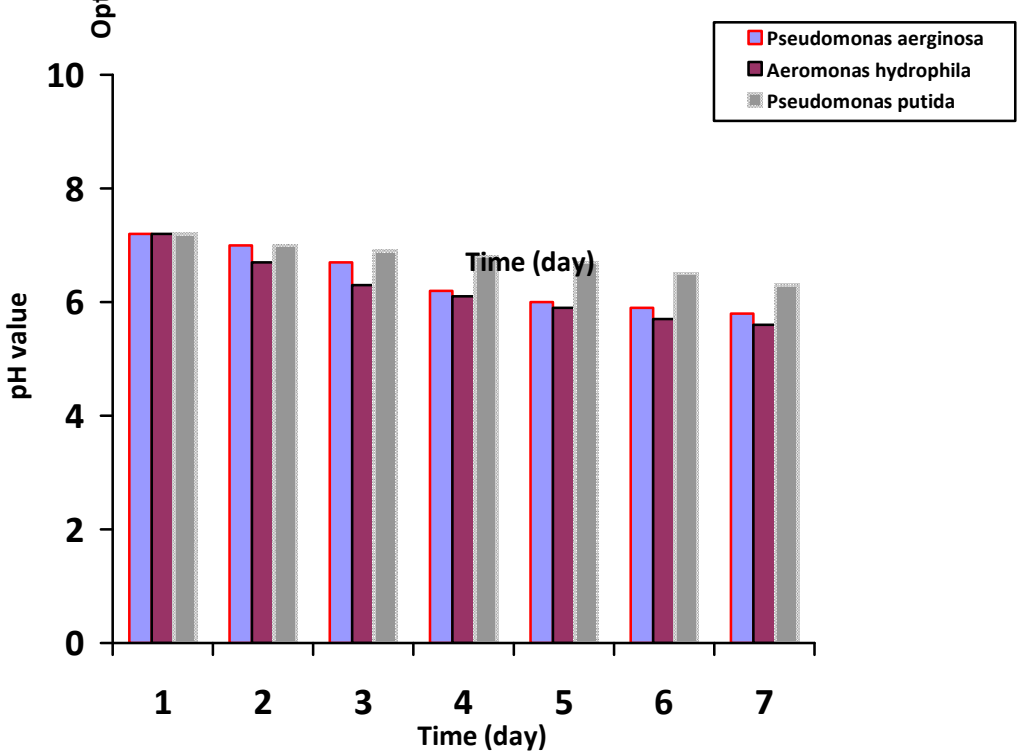


Fig. (2) . pH values of isolated bacterial isolates during incubation period

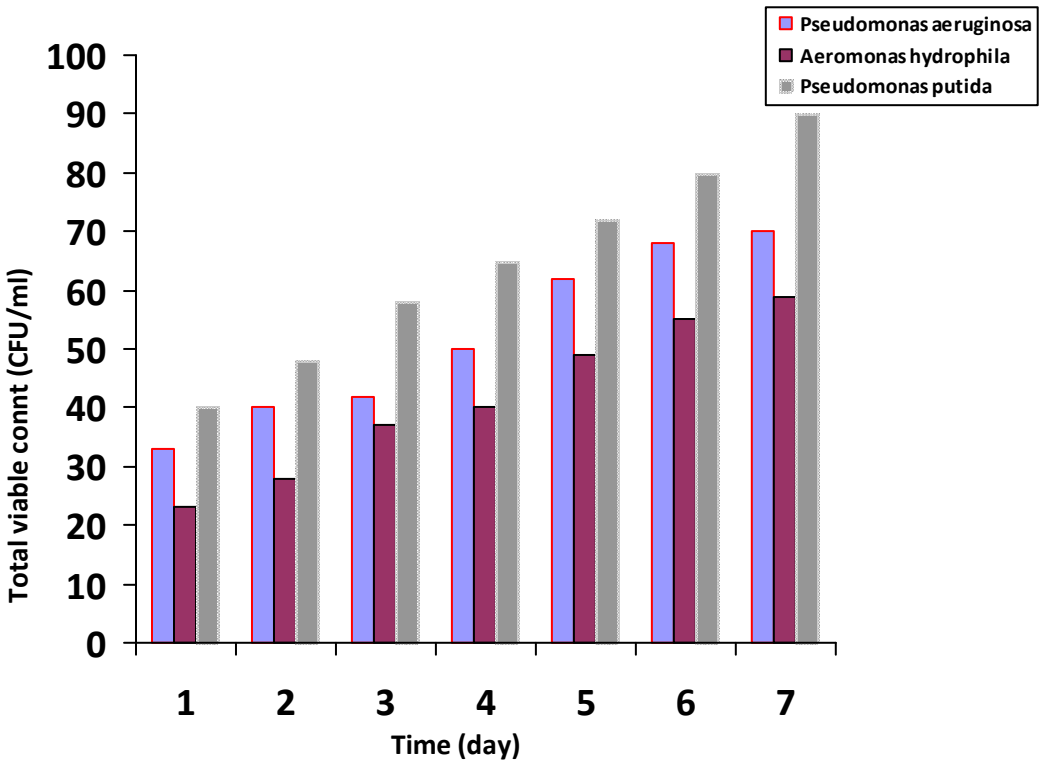


Fig.(3): Growth profiles of *Pseudomonas aeruginosa* , *Aeromonas hydrophila* , and *Pseudomonas putida* according to total viable count (CFU/ml) during incubation period.

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