Egypt. J. Exp. Biol. (Bot.), 13(3): 19 – 30 (2017)
DOI:10.5455/egyjebb.20170131064321

RESEARCH ARTICLE

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Bioremediation of the nematicide oxamyl by *Enterobacter ludwigii* isolated from agricultural wastewater

ABSTRACT:

Oxamyl is an important carbamate nematicide that is used for the control of nematodes in many economic crops in Egypt. It is characterized by high acute toxicity to mammals and aquatic organisms. Microbial degradation is the main approach controlling the environmental contamination with oxamyl. In this current study, using enrichment technique, oxamyl degrading bacterium was isolated from agricultural drainage ditches of oxamyl-treated fields (Fayoum, Egypt). The isolated bacterium was identified as *Enterobacter ludwigii* based on the biochemical characterization and 16S rDNA gene sequencing. An axenic culture of *E. ludwigii* was grown in minimum salt medium enriched with oxamyl as sole carbon and nitrogen source. Moreover, the factors affecting on oxamyl degradation were investigated. The maximum capability of oxamyl degradation was achieved at 200 ppm of oxamyl within 6 days at pH value 7.0 and temperature 37°C. In conclusion, this study clarified the notable capability of *E. ludwigii* for the degradation of oxamyl from contaminated agricultural wastewater.

KEY WORDS:

Enterobacter ludwigii, oxamyl, nematicides, biodegradation, 16S rDNA.

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ARTICLE CODE: 03.02.17

INTRODUCTION:

Intensive use of pesticides has resulted in severe contamination and destruction of biodiversity and ecological systems. The worldwide annual consumption of pesticides has been estimated to be about two million tons (Abhilash and Singh, 2009). Increasing use of pesticides in agriculture and domestic activities for controlling pests is polluting the environment progressively (Memon *et al*., 2008). Most herbicides applied to crops are absorbed by plants or degraded in the soil, but small fractions might move to streams in overland runoff, near surface flow, or subsurface drains or they infiltrate slowly to ground water (Chapalamadugu and Chaudhry, 1992; Battaglin *et al*., 2003). Pesticides can negatively interfere with some vital processes in the microbial cells (De Lorenzo *et al*., 2001). At the same time, the impact of pesticides on aquatic environments could arise from their degradation products that might be more harmful than the original compounds (Thurman *et al*., 1992; Battaglin *et al*., 2003).

Carbamates are intensively used as pesticides in agriculture because of their broad spectrum of activity. Stability of carbamates decreases quite in aquatic environments, so these compounds are rarely detected in freshwater systems. They can only persist for between 4 and 12 weeks, depending on pH, temperature, and other constraints (Albanis *et al*., 1998). On the contrary, carbamates were detected in rivers and streams of the Caribbean island of Martinique (Bocquene´ and Franco, 2005). Twelve types of carbamate groups were detected in 85.5% of the Chinese kale samples from the local consumer market (Apilux *et al*., 2015). Oxamyl is an important carbamate nematicide that used for the control of nematodes in carrots, parsnips, potatoes and sugar beet crops (Osborn *et al.*, 2010).

Oxamyl (Fig. 1) is used in a wide range of agricultural situations; it is active and systemic as a nematicide (Tomlin, 2002; Minnis *et al*., 2004) or an insecticide (Mowry, 2005). Besides, oxamyl can be mixed with *Bacillus thuringiensis* or sesame-oil-cake to inhibit the growth of the nematode *Meloidogyne incognita* (El-Sherif *et al*., 2007). Oxamyl is defined as a highly toxic compound that have acute toxic effect on human

(Tomlin, 2002) and aquatic organisms (Sørensen *et al*., 2008). Oxamyl toxicity is attributed to their destructive effect on DNA, in addition to the suppression of acetylcholinesterase leading to the accumulation of the acetylcholine which in turn, causes neurotoxic symptoms (Du *et al*., 2008).

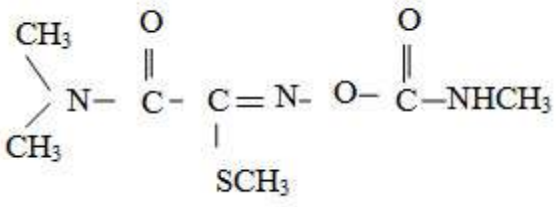

Fig. 1. Chemical structure of oxamyl (Methyl N'N'-dimethyl-N-[(methylcarbamoyl) oxy]-1-thiooxamimidate)]

The fate of pesticides in environment depends on the physical and chemical properties of the pesticides and the microbial activities in the soil. Microorganisms can degrade a wide variety of synthetic chemicals. Several strains of bacteria as *Pseudomonoas putida*, can use substances such as phenol or naphthalene, which could be a skeletal structure of insecticides (Stainer *et al*., 1966). Plangklang and Reungsang (2013) reported that, microbial population of carbofuran-degrading bacteria increased in soils with much years of carbofuran application compared to soils having little years of carbofuran exposure. Moreover, Rozo *et al*. (2013) purified 48 isolates capable of degrading carbofuran in soil with 8 years of carbofuran. Because of high mammalian toxicity of such compounds and their widespread and extensive use, microbial degradation of pesticides is of particular interest (Singh *et al*., 2004; Reyad *et al*., 2014; Essa *et al*., 2016).

Biodegradation and bioremediation are processes that are based on the conversion or metabolism of pesticides by microorganisms. The difference between these two is that, the biodegradation is a natural process, whereas the bioremediation is a technology. In bioremediation, microbes were used to degrade pesticides *in situ* (Singh, 2008). Photoautotrophic microorganisms such as microalgae and cyanobacteria have potential to remove various pesticides (Ibrahim and Essa, 2010), heavy metals (Ibrahim, 2011) and textile dyes (Parikh and Madamwar, 2005). Microbial bioremediation is an efficient strategy due to its high efficiency, low cost, and eco-friendly nature (Rajendran *et al*., 2003; Talley, 2005; Wasi *et al*., 2008, 2011a&b). The addition of microbial cultures capable of breaking pesticides down or so-called bioaugmentation techniques, is reported to be an effective bioremediation pathway for improving pesticide removal in contaminated soils and water that lack any indigenous microbial activity (Parameswarappa *et al*., 2008; Marecik *et al*., 2008).

The microbially mediated breakdown of pesticides is more important than other physical and chemical degradation. Chemical treatment processes often yield insufficient results if the water contains high amounts of non-biodegradable (refractory) organic substances (Samet *et al*., 2006). Conversely, biotic degradation proceeds either directly (through mineralization, polymerization or co-metabolism) or indirectly, through secondary effects of microbial activity altering soil pH and redox conditions (Bollag and Liu, 1990).

Biodegradations of carbamate pesticides by different bacteria were demonstrated by several authors (Doddamani and Ninnekar, 2001; Barragán-Huerta *et al*., 2007). Some bacteria isolated from soils with prior history belong to the genera *Enterobacter*, *Pseudomonas*, *Verinia*, *Flavobacterium*, *Flexibacterium* involved in the biodegradation of some carbamate pesticides as carbofuran (Chaudhry and Ali, 1988; Nawaz *et al.,* 2011; Mohanta *et al*., 2012; Plangklang and Reungsang, 2013). Moreover, Konstantina *et al*. (2016) isolated four oxamyl-degrading bacterial strains from an agricultural soil belong to the genus *Pseudomonas* that exhibiting enhanced biodegradation of oxamyl.

In Egypt, oxamyl nematicide is used in a wide range of agricultural situations. So, the aim of this work was directed to (i) isolate oxamyl tolerant bacteria from agricultural wastewater in Fayoum Governorate, Egypt, (ii) investigate the optimum condition of oxamyle biodegradation.

MATERIAL AND METHODS:

Oxamyl (99.6%) was purchased from Riedel-de Haën (Seelze, Germany). All other chemicals purchased are of analytical grade from Fluka (Switzerland).

For the isolation of bacteria from agricultural wastewater and oxamyl degradation studies, a minimal salt medium (MSM) was used as stated by Cycoń *et al.* (2013) with minor changes. The MSM was consisting of 1.5 (g/l) KH_2PO_4; 2.0 (g/L) $(NH_4)_2SO_4$; 1.5 (g/L) Na_2HPO_4; 0.01 (g/L) $CaCl_2 .2H_2O$; 0.2 (g/L) $MgSO_4 .7H_2O$; 0.001 (g/L) $FeSO_4.7H_2O$. Using 2M NaOH, pH of the medium was adjusted to 7.0 ± 0.1. Oxamyl was added to MSM medium after sterilization. For solid medium, 2% (w/v) agar was added to the same medium for the preparation of solid MSM. An aqueous solution of oxamyl (1000 ppm in sterile dH_2O) was diluted to the required concentrations for the degradation studies.

The agriculture wastewater sample collected from El-Batts drain, Fayoum, Egypt (500 ml) was centrifuged at 10,000 rpm for 10 min and the pellet was suspended in 5 mL dH_2O. A five milliliter of the bacterial suspension was used to inoculate 45 ml liquid MSM enriched with 100 ppm oxamyl and incubated at 30^oC inside shaking incubator at 120 rpm (GFL orbital shaker model 300s). After 48 hrs, aliquots were sub-cultured in fresh oxamyl containing medium. This

step was repeated 5 times and the final culture was diluted and plated on oxamyl agar plates. The developed colonies were repeatedly streaked on oxamyl agar plates for the isolation of pure bacterial cultures. A pure culture of the most tolerant strain (AOX) that can grow under elevated levels of oxamyl up to 400 ppm was chosen for this study.

The motility and Gram stain tests were conducted for the bacterial isolate AOX. While the biochemical characterization was carried out according to Selim *et al*. (2016) using commercially available multi-test identification systems API (BioMérieux, France). Test strips were inoculated and incubated according to the instructions provided. Sterile 0.85 % saline solution was used as a negative control. APIWEB software was used for identification.

To confirm the biochemical identification of the bacterial isolate AOX, 16S rDNA gene sequencing technique was performed. According to Essa (2012), the genomic DNA was extracted and the amplification of the 16S rDNA gene was conducted using forward primer (F1; AGA GTT TGA TCC TGG CTC AG) and reverse primer (R1; GGT TAC CTT GTT ACG ACT T). The PCR mixture and PCR program were carried out as described by Essa *et al*. (2016). The amplified fragments after purification were sequenced at GATC Biotech, Constance, Germany. The DNA Sequences were aligned at NCBI Data Base (www.ncbi.nlm.nlh.gov). Based on 16S rRNA gene sequences of AOX and some strains phylogenetically close to the isolated strain, a phylogenetic tree was constructed using TREEVIEW software (1.6.6).

To investigate the effect of oxamyl concentrations on the growth of the bacterial AOX strain, 50 ml MSM supplemented with different oxamyl concentrations (50, 100, 200, and 300 ppm) was inoculated by 5 ml bacterial suspension (OD600 = 0.6). During the incubation of the culture on a rotary shaker (120 rpm) at 30°C, the bacterial growth was assayed spectrophotometrically (Shimadzu UV-Visible recording spectrophotometer model UV-160A) by measuring the cultural optical density at 600 nm at 24 hr intervals over 14 days. At the same time, the protein content of the bacterial cultures was determined using Bradford assay (Bradford, 1976). The effect of pH value and temperature on the bacterial growth and rate of oxamyl degradation was investigated. Cultures supplemented with 200 ppm oxamyl as a sole carbon and nitrogen source at different pH values (5.0, 7.0, and 9.0) and different temperatures (20, 30, and 37^{o}C) were incubated as mentioned above. All the experiments were done in triplicates and cell growth was determined spectrophotometrically as mentioned above. In order to measure the abiotic degradation of oxamyl, MSM enriched with the same oxamyl concentrations in absence of bacteria were prepared and incubated under the same conditions.

High performance liquid chromatography (HPLC) was used to measure the residual oxamyl according to (Osman *et al*., 2009). Fifty milliliter methanol (90%) was added to 20 mL cell free culture and the mixture was filtrated and extracted twice with 50 mL CH_3Cl. Then the solution was concentrated to 1 mL. A Hewlett-packard, USA serial 6890 gas chromatograph equipped with electron detector (ECD, Radioisotope Nuclide 63Ni) and HP PAS-1701 column 25 m length x 0.32 mm x 0.52 thickness. Pure nitrogen was used as carrier gas (2 mL/min). Detector, injector and column temperature was 250, 240 and 225°C, respectively. The oxamyl degradation rate was calculated according to Lin *et al*. (2008) by the following formula:

$$A = [C_a - C_b / C_a] \times 100$$

Where (A) is the percentage of oxamyl degradation, (C_a) is the concentration of oxamyl (mg/l) in absence of bacteria; (C_b) is the concentration of oxamyl (mg/l) in presence of degrading strain.

Statistical analysis:

The data presented are the mean values of three replications. Standard errors were calculated for all the values using MS Excel 2007.

RESULTS:

The bacterial species capable of degrading oxamyle was isolated from agricultural drainage ditches in Fayoum Governorate, Egypt, using enrichment technique. The bacterial isolate AOX was the most tolerant strain against high levels of Oxamyl (300 ppm).

A variety of morphological and biochemical assays were carried out to have a comprehensive view of phenotypic characteristics of the bacterial isolate AOX as shown in table 1. AOX isolate was gram negative motile non-spore forming rods. This isolate demonstrated positive results with β-galactosidase, arginine dihydrolase, ornithine decarbolase, tryptophane deaminase, amylase, N_2 gas production and acetone production. Meanwhile, negative results were obtained for the following tests: lysine decarboxylase, urease, gelatinase, catalase, lipase, cytochrome oxidase, Nitrate and nitrite reduction, H_2S production and indole production. Simultaneously, the AOX isolate showed the capability to utilize glucose, sucrose, mannitol, inositol, rhamnose, melibiose, amagdalin, arabinose, starch and citrate as carbon sources. The AOX isolate was identified as *Enterobacter ludwigii* using 16S rDNA gene sequencing technique with maximum homology of 96% to *Enterobacter ludwigii*. The phylogenetic tree of the oxamyl degrader bacterial strain AOX and related bacterial species based on the 16S rDNA sequence was provided in figures 2 and 3. It can be clearly seen that, the oxamyl degrader bacteria was included in the genus *Enterobacter* and closely related to the species *ludwigii*.

Table 1. Biochemical characterizations of the oxamyl degrading bacterial isolate AOX

Reaction	Result	Reaction	Result
Morphological characters		**Fermentation of sugars**	
Cell shape	Rod	Amygdalin	+ve
Endospore formation	-ve	Arabinose	+ve
Gram staining	-ve	Citrate utilization	+ve
Motility	+ve	Glucose	+ve
Biochemical characters (Enzyme profile)		Inositole	+ve
Amylase	+ve	Mannitol	+ve
Arginine dihydrolase	+ve	Melibiose	+ve
Catalase	-ve	Rhamnose	+ve
Cytochrome oxidase	-ve	Sorbitol	+ve
β-galactosidase	+ve	Starch	+ve
Gelatinase	-ve	Sucrose	+ve
Lipase	+ve	**Other tests**	
Lysine decarbolase	-ve	Acetone production	+ve
Nitrate reduction	-ve	H_2S production	-ve
-To nitrite	-ve	Indole production	-ve
-To N_2 gas	+ve		
Orenthine decarbolase	+ve		
Tryptophane deaminase	+ve		
Urease	-ve		

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AOX   84  TGCCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGA  143
          |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 104  TGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGA  163
AOX   144  CCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGT  203
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 164  CCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGT  223
AOX   204  AGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCC  263
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 224  AGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCC  283
AOX   264  ACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC  323
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 284  ACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC  343
AOX   324  AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAA  383
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 344  AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAA  403
AOX   384  GTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGC  443
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 404  GTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACCCGC  463
AOX   444  AGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT  503
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Sbjct 464  AGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT  523
AOX   504  AATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC  563
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Sbjct 524  AATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC  583
AOX   564  CCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGT  623
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 584  CCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGT  643
AOX   624  AGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC  683
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 644  AGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC  703
AOX   684  GGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA  743
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Sbjct 704  GGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA  763
AOX   744  TACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGG  803
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 764  TACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGG  823
AOX   804  CTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCCGCAAGGTTAAAACT  863
          |||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct 824  CTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGG-CCGCAAGGTTAAAACT  882
AOX   864  CAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACG  923
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 883  CAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACG  942
AOX   924  CGAAGAACCTTACCTACTCTTGACATCCAGAGGACTTTCCAGAGATGGATT-GTGCCTTC  982
          |||||||||||||||||||||||||||||||| ||||  ||||||||  || ||||||||
Sbjct 943  CGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTC  1002
AOX   983  GGGAACTCTGAGACAG-TGCTGCAT-GCTGTCGTCAGCTCGTGT-GTGAA-TGT-GGAT-  1036
          |||||||||||||| |||||||| |||||||||||||||| ||||| ||| || |
Sbjct 1003 GGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT  1062
AOX   1037 -AGTCCCGCAACGAGCGCA-CCCT-ATC-TT-GT-GC-AGCG-TC-GGC-GG-A-CTCA-  1083
          |||||||||||||||| |||| ||| || || || |||| || ||| || | ||||
Sbjct 1063 AAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAA  1122
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AOX    1084  -G-A-ACTGC-A-TGATA--CTG-AGGA-G-TGGG-ATGACGTCA-GTCATC-TGTCC-T 1129
| | |||||| | |||||  ||| |||| | |||| |||||||||| |||||| || || |
Sbjct  1123  AGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCT  1182
AOX    1130  -AC-A-TAGG-CTAC-CACG-GCTACA  1150
|| | |||| |||| |||| ||||||
Sbjct  1183  TACGAGTAGGGCTACACACGTGCTACA  1209
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Fig. 2. Partial DNA sequences of the 16S rDNA gene of the bacterial strain AOX isolated from agricultural wastewater and the corresponding gene of *Enterobacter ludwigii. Enterobacter ludwigii* strain: EN-119 = DSMZ 16688 = CIP 108491 16S ribosomal RNA, complete sequence

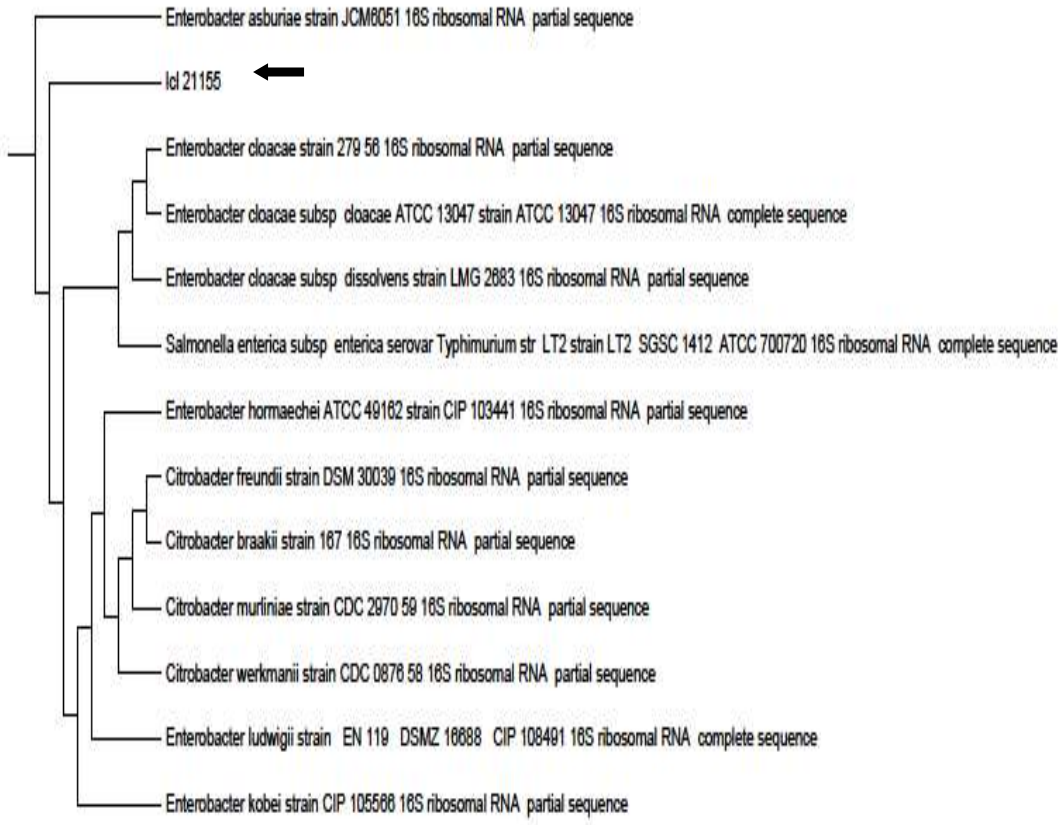


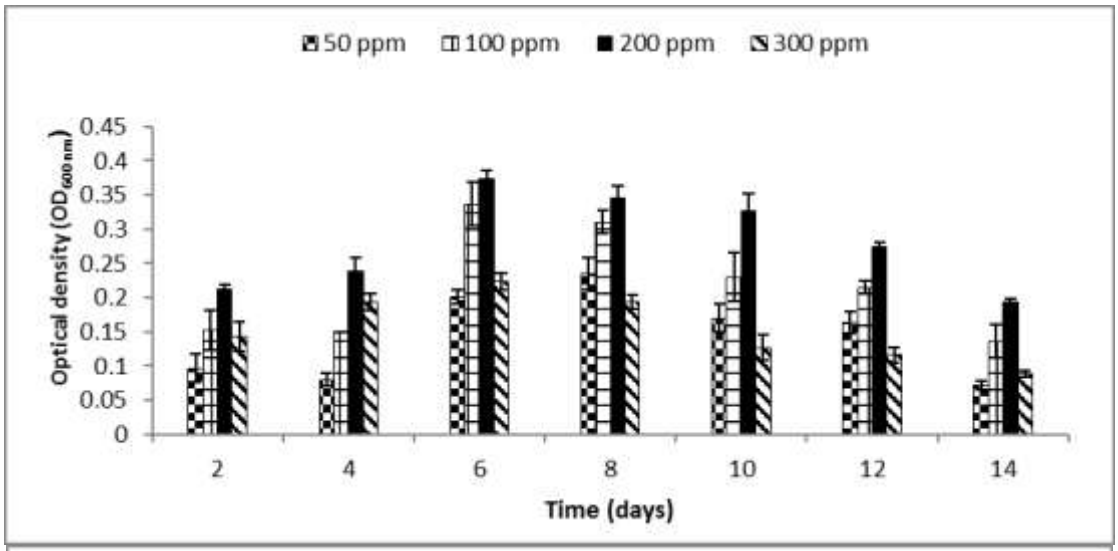
Fig. 3. Phylogenetic dendrogram obtained by analysis of 16S rDNA sequences, showing the position of bacterial strain (AOX) among phylogenetic neighbors. The black arrow indicates the position of AOX strain.

In order to specify the optimum growth conditions of *Enterobacter ludwigii*, the effects of oxamyl concentration, temperature and pH value were investigated. Data in figure 4 A&B showed the effect of oxamyl concentrations (50, 100, 200, and 300 ppm) as a sole carbon source on the growth of *Enterobacter ludwigii*. The maximal growth (OD: 0.373) was recorded at 200 ppm of oxamyl after 6 days of incubation, while the highest protein content (86 mg/L) was observed after 6 days at the same concentration of oxamyl. Above or beyond this concentration, an obvious reduction in growth and protein content of the bacterial culture was recorded.

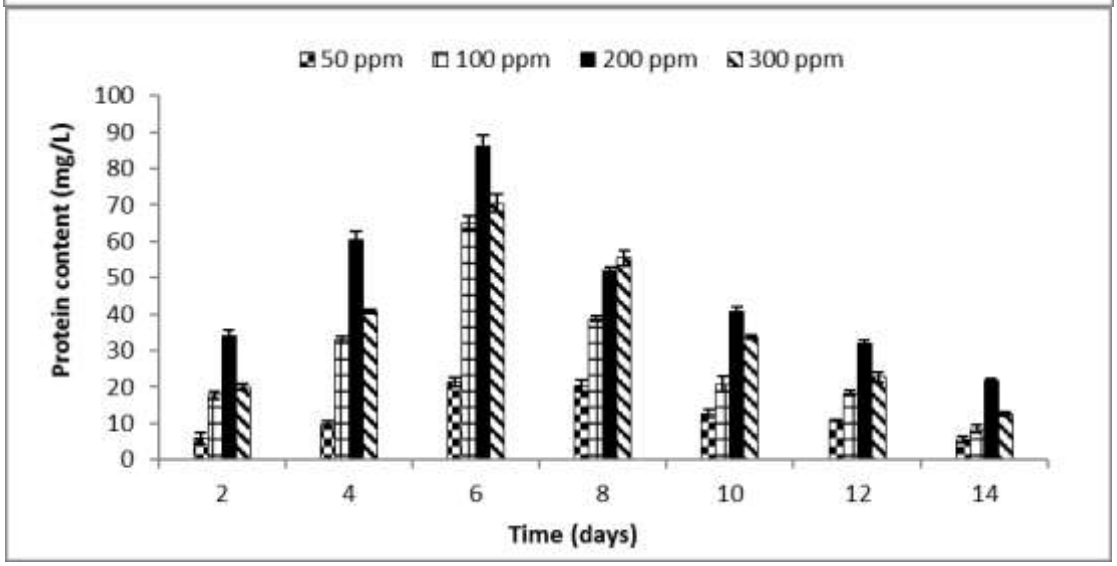
Data in figure 5 A&B showed the effect of different temperature values on the growth of *Enterobacter ludwigii.* Oxamyl (200 ppm) was used as sole carbon source. The maximum bacterial optical density (0.396) and protein content (55.8 mg/L) were demonstrated at 37°C after 6 days. At temperatures (20°C or 37°C), a clear inhibition in growth (OD: 0.298 & 0.349) and protein content (20.6 & 41.7 mg/L), was recorded, respectively.

Data in figure 6 A&B showed the effect of pH values on the growth of *Enterobacter ludwigii.* Oxamyl (200 ppm) was used as sole carbon source. The maximum bacterial optical density (0.376) and protein content (60.8 mg/L) were obtained at pH 7.0 after 6 days. In the meantime, the recorded optical density (0.167 & 0.29) were obtained at pH values (5.0 and 9.0) with protein content (17 & 52.27 mg/L), respectively.

Data in table 2 verified the effects of temperature and pH values on biodegradation rate of oxamyl, where the highest degradation percentages (67.4% and 83.5%) were demonstrated at 37°C and pH 9, respectively, after 6 days of incubation. Oxamyl degradation was significantly reduced at pH 5.0 and temperature 20°C.

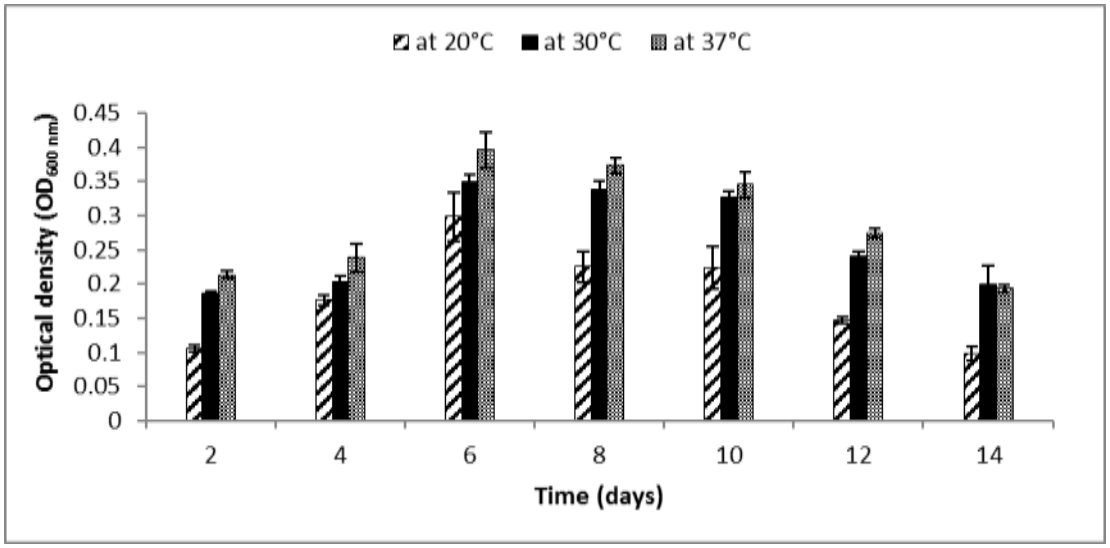


A

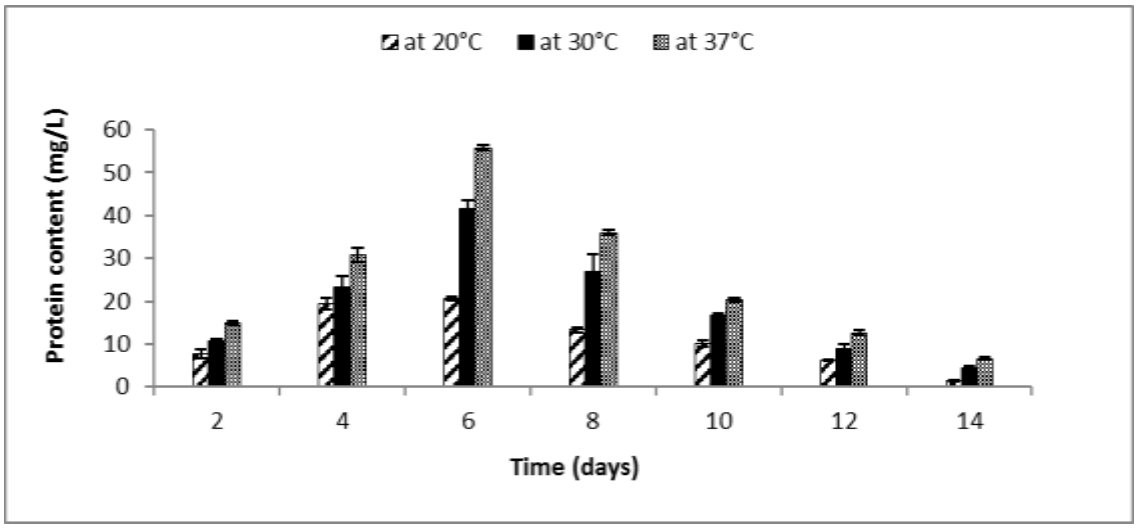


B

Fig. 4. Effect of different oxamyl concentrations on growth of *E. ludwigii*. (A) In terms of optical density ($OD_{600\ nm}$); (B) represents the protein content (mg/L). Oxamyl (50, 100, 200, and 300 ppm) was used as sole carbon source. Data are the means of three replicates and error bars represent the standard errors of the means.

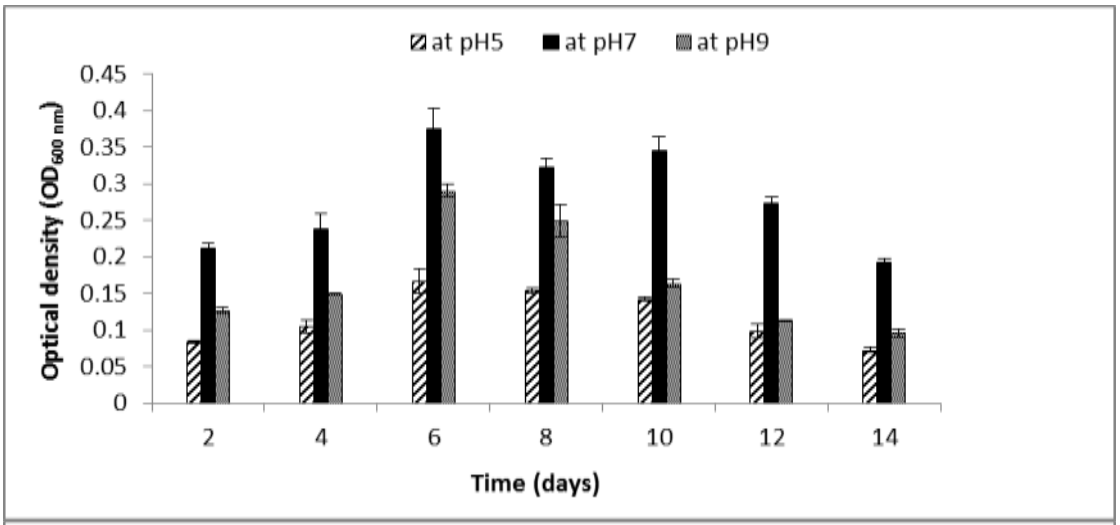


A

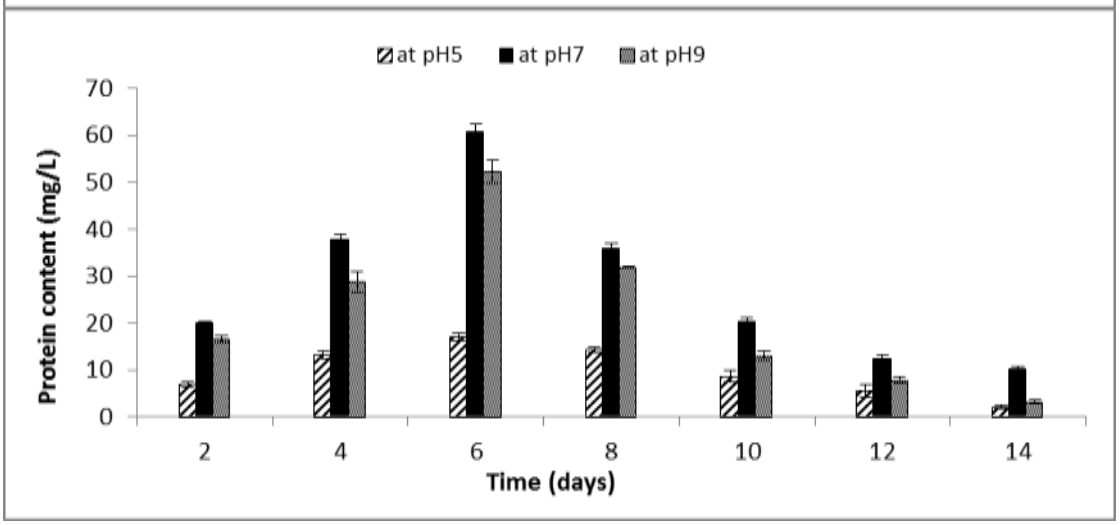


B

Fig. 5. Effect of different temperature degrees on growth of *Enterobacter ludwigii*. (A) In terms of optical density ($OD_{600\ nm}$); (B) represents the protein content (mg/L). Oxamyl (200 ppm) was used as sole carbon source. Data are the means of three replicates and error bars represent the standard errors of the means.



A



B

Fig. 6. Effect of pH values on growth of *Enterobacter ludwigii*. (A) In terms of optical density ($OD_{600\ nm}$); (B) represents the protein content (mg/L). Oxamyl (200 ppm) was used as sole carbon source. Data are the means of three replicates and error bars represent the standard errors of the means.

Table 2. The percentage of oxamyl removal by *Enterobacter ludwigii* under different temperatures and pH values after 6 days of incubation. The initial concentration was 200 ppm.

Treatment		Retention time (min)	Residual (ppm)	Removal (%)
pH value	5	2.106	177.1	16.5
	7	2.106	47.2	77.7
	9	2.114	116.6	45
Temperature	20°C	2.1	122.5	42
	30°C	2.1	57.4	73.1
	37°C	2.106	47.8	77.4

DISCUSSION:

Excessive use of pesticides has resulted in severe contamination and a destruction of the ecological systems and biodiversity. A great portion of pesticide residues in the soil are transported into water and get broken down to more or less harmful substances (Memon *et al*., 2008; Thengodkar and Sivakami, 2010). The microbial mediated breakdown of pesticides is more important than the physical and chemical degradation. The role of bacteria in the biodegradation and detoxification of the toxicants is well demonstrated by Wasi *et al.* (2011 a&b).

The present investigation was conducted to study the survival and tolerance of bacteria to elevated concentrations of oxamyl as well as their efficiency for the mineralization of this compound. The bacterial strain designated as AOX was the most dominant strain in the agricultural drainage ditches (Fayoum, Egypt). It was chosen due to its capability to persist under elevated concentrations of oxamyl (200 ppm). In fact, certain bacterial populations can exist in the agricultural wastewater under high levels of pesticide contamination. Such strains may have the potentiality to degrade these toxic compounds (Essa *et al*., 2016). A variety of morphological and biochemical assays were carried out to have a comprehensive view of the phenotypic and physiological characteristics of the oxamyl tolerant isolate AOX. Simultaneously, it was identified as *Enterobacter ludwigii* using molecular technique.

The growth responses of *Enterobacter ludwigii* in terms of optical density and protein content was recorded in MSM amended with different concentrations of oxamyl. Several studies have shown that *Enterobacteriaceae* may have beneficial effects on plant development when they are associated with plants (Taghavi *et al*., 2009). They may improve plant growth via nitrogen fixation, suppression of plant pathogens and production of growth promoting molecules (Kämpfer *et al*., 2005; Madhaiyan *et al*., 2010). Various *Enterobacter* members, *Enterobacter cloacae* and *Enterobacter ludwigii*, are known for their potential pathogenicity to humans (Paauw *et al*., 2008). In fact, few reports on *E. ludwigii* are available, but it has been reported as a plant associated bacterium with plant growth promoting, biocontrol ability and petroleum degradation (Shoebitz *et al*., 2009; Taghavi *et al*., 2009; Madhaiyan *et al*., 2010; Yousaf *et al*., 2011).

Oxamyl belongs to the carbamates group of pesticides is used for control of chewing and sucking insects, spider mites and nematodes in many crops. Within the soil, oxamyl is degraded via hydrolysis to its non-toxic oximino metabolite (Bromilow, 1973). The potentiality of oxamyl to be leached into ground water was attributed to its high-water solubility and poor soil sorption (Gianessi and Marcelli, 2000). The present study demonstrated a remarkable tolerance capability of *Enterobacter ludwigii* isolated from the agricultural wastewater to high concentrations of oxamyl as well as a high potentiality for the degradation of this pesticide. Previous studies showed that some carbamate pesticides were efficiently degraded by various bacterial genera such as *Enterobacter, Pseudomonas, Verinia, Flavobacterium, Flexibacterium* (Mohanta *et al*., 2012; Plangklang and Reungsang, 2013). Furthermore, some oxamyl tolerant bacteria were isolated from agricultural canals in Texas, USA. These isolates displayed diverse phenotypes and could use many organic substrates (Aguirre and Lowe, 2010).

Bacteria have several mechanisms that allow them to tolerant or resistant toxic pollutants. One of these strategies is the release of different degrading enzymes that can metabolize the toxic compound (Talaro, 2008). Microbial degradation takes place when microorganisms metabolize the active ingredient and the degradation products to access the carbon as an energy source. The frequent application of specific pesticides in the same field site could lead to rapid dissipation of these compounds due to the phenomenon of enhanced microbial degradation. Such these soils have been used for the isolation of pesticide degrading bacteria (Castellanos *et al*., 2013). Biodegradation of carbamate insecticide by bacteria were demonstrated by several

workers (Barragán-Huerta *et al*., 2007; Nawaz *et al*., 2011; Tien *et al*., 2013).

In 2010, Osborn and coworkers showed that the recurrent utilization of oxamyl in agricultural soils has led to an enhancement of its degradation by specific bacterial strains that demonstrated a high capability to utilize this pesticide as a sole carbon source. Similarly, Chanika *et al*. (2011) highlighted the potentiality of *Pseudomonas putida* for the degradation of oxamyl and carbofuran. Recently, a pseudomonad strain having the ability to grow in the presence of carbosulfan pesticide was isolated from cultivated soil in Bangladesh. This bacterial strain demonstrated high ability to grow in the presence of different concentrations of carbosulfan pesticide (Sharif and Mollick, 2013). In fact, the capability of bacteria to degrade carbamate pesticides depends on the presence of specific genes such as carbofuran hydrolase, carbaryl hydrolase, and oxamyl hydrolase have been isolated and identified (Tomasek and Karns, 1989; Hashimoto *et al*., 2002; Rousidou *et al*., 2016).

The current study clarified the optimum conditions of the bacterial growth and oxamyl degradation by *E. ludwigii*. The obtained results demonstrated a proportional relationship between oxamyl concentration and bacterial growth that was attributed to the capability of this strain to degrade and utilize oxamyl as a growth substrate. Microbial degradation of pesticides can be affected with different factors such as temperatures, pH level, soil moisture, and aeration. The present study showed that the maximum growth of *E. ludwigii* and the highest degradation rate of oxamyl were achieved at pH 7.0 and at 37˚C. These findings are in harmony with our previous study (Essa *et al*., 2016) where *P. aeruginosa* isolated from contaminated wastewater demonstrated the greatest degradation rate of diazinon at pH value 7.0 and temperature 30°C. Similarly, Fang *et al*. (2010) showed that the growth of *Enterobacter* sp. on dibutyl phthalate increased rapidly by increasing the temperature and the maximum growth and degradation rate was achieved at 35˚C and pH 7.0. In the same way, Chino-Flores *et al.* (2012) identified the optimum pH value for the degradation of some organophosphorus pesticides by *Enterobacter* sp. in minimum salt medium at 7.0. Similarly, Naqvi *et al*. (2013) clarified that the highest rate of carbfuran degradation by *Pseudomonas aeruginosa* was recorded at pH 7.5 and at 40 °C.

In conclusion, an oxamyl tolerant bacterial strain was isolated from agricultural drainage ditches by enrichment technique. According to the biochemical and molecular characterization, this strain was identified as *Enterobacter ludwigii*. This strain showed a high capability to utilize oxamyl as a sole carbon and nitrogen source. A remarkable rate of oxamyl degradation was achieved at pH value 7.0 and temperature 37°C within 6 days. Although *E. ludwigii* is considered as opportunistic pathogen, it could be used as a source of some pesticides degrading enzymes that may be employed for the abolishment of high levels of oxamyl and other pesticides from agricultural wastewater.

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المعالجة الحيوية للمبيد النيماتودي "الأوكساميل" باستخدام بكتيرة "إنتيروباكتر لودفيجي" المعزولة من مصارف المياه الزراعية

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يعتبر الأوكساميل أحد المبيدات الحشرية التي تستخدم عادة من أجل السيطرة على الديدان الخيطية في العديد من المحاصيل الاقتصادية في مصر. يتميز هذا المبيد بتأثيره السام على الثدييات والكائنات المائية. إن التحلل الميكروبي للأوكساميل يعد هو النهج الرئيسي للسيطرة على التلوث البيئي بهذا المبيد. في هذه الدراسة تم عزل أحد أنواع البكتيريا ذات القدرة الملحوظة على تحمل تركيزات مرتفعة من المبيد من قنوات الصرف الزراعي (الفيوم، مصر). تم تعريف هذه البكتريا علي إنها ***Enterobacter ludwigii*** إستنادا للخصائص المورفولوجية والبيوكيمائية والتسلسل الجيني 16SrDNA.

لقد تم زراعة هذه البكتريا على بيئات معدنية مثراة بالأوكساميل كمصدر وحيد للكربون والنيتروجين. كما تناولت هذه الدراسة تأثير تركيز الأوكساميل وكذلك درجة الحرارة والرقم الهيدروجيني على نمو البكتيريا ومعدل التحلل الميكروبي لهذا المبيد. وقد تحققت القدرة القصوى لتحلل الأوكساميل عند تركيز 200 جزء في المليون والرقم الهيدروجيني 7.0 ودرجة الحرارة 30° م في غضون 6 أيام. وقد أوضحت هذه الدراسة قدرة بكتيريا *E. ludwigii* على تكسير مبيد الأوكساميل بكفاءة عالية من مياه الصرف الزراعي الملوثة بهذا المبيد.