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CONSTRUCTION OF EFFECTIVE BACTERIAL ISOLATES FEATURING PHOSPHATE DISSOLUTION AND PESTICIDES BIODEGRADATION VIA INTRAGENIC AND INTRASPECIFIC CONJUGATION

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ABSTRACT

The present study aimed to induce some highly phosphate dissolving and pesticides degrading bacterial isolates via conjugation technique. Two hundred bacterial isolates (collected from rhizosphere soils cultivated with different crops in different regions in Egypt, previously exposed for many pesticides for long time). In addition two bacilli strains were used. The efficiency of phosphate dissolving showed significant differences. Eight isolates and the two bacilli strains were chosen for conjugation experiment, while they appeared different responses to eight types of antibiotics and three different pesticides . These isolates were found to be belonging to *Pseudomonas* sp. Two types of conjugation were conducted i.e., intrageneric (among *Pseudomonas* isolates) and intraspecific (between two *bacillus* megaterium strains). The results showed that 16 from all the 135 selected transconjugants proved to have the ability to release phosphours amounts more than the highest parental isolate (Km 15). The majority of these transconjugants also proved to have the ability to degrade all doses of the applied pesticides with some minor exceptions. The biochemical analysis results showed that there was one band (66.00 K.D) that may be associated with phosphate solubilizaiton activity.

Keywords: *Pseudomonas- bacillus*-phosphate-pesticides-biodegradation-intragenic and intraspecific conjugation.

INTRODUCTION

Phosphorus is an important nutrient required for the basic process of life. Also, it is an essential nutrient for plant growth, whereas it functions as an agent of energy transfer, and a deficiency of available phosphorus is more likely to limit reproduction and productivity than any other materials, except water. The conversion of phosphorus to insoluble form is one of the most important problems of phosphate fertilization in Egypt. Alkalinity of the Egyptian soils rapidly converts soluble forms of inorganic P-fertilizer applied to the soils, to less available forms, mainly as a complex precipitated form of $Ca_3(PO_4)_2$. Some soils microorganisms play an important role in supplying the grown plants with available forms of phosphorus through production of organic, inorganic acids and Co_2 (Hellstén *et al.*, 2005). They increase the soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Alexander, 1977).

The widespread agricultural use of pesticides resulted chemical entering soil and water ecosystems. In Egypt, the use of pesticides has become an integral and economically essential part of agriculture. Many problems arise from the use of synthetically produced pesticides. Biodegradation is the phenomenon where by which soil-applied pesticides are rapidly degraded by a population of microorganisms that had adapted due to previous exposure to the pesticides. These microorganisms are capable of utilizing various pesticides as the sole source of nitrogen, carbon and phosphorus. Also, microorganisms may degrade a pesticide because it is toxic to them (Digrak and Ozel, 2002 and Kao *et al.* 2004).

This study aimed to construct highly phosphate dissolving and pesticides degrading bacterial isolates via DNA transfer technique (conjugation).

MATERIALS AND METHODS

A. MATERIALS:

A.1. Microorganisms:

A.1.a. Bacterial isolates:

Two hundred bacterial isolates were collected from the rhizosphere of different plants from different regions that were previously exposed to many pesticides. Eight isolates were selected and used in this study and were nominated, Gm15 , Kf1, Ds1, Gs7, Km10, Km15, Gm11 and Kw1.

A.1.b. Bacterial strains:

Two bacterial strains were also selected; *Bacillus megaterium* var. *phosphaticum* "Phosphobacterin", and *Bacillus megaterium* A.H. Both strains proved to be effective in dissolving the precipitated forms of phosphate.

A.2. Media:

Four different media were used; Luria medium (LB) (Sambrook *et al.*, 1989), Nutrient broth medium (NB) (Difco, 1953), King's B medium: (Belal, 1996) and Bunt and Rovira medium (1955) as modified by Abdel-Hafez (1966).

A.3. Antibiotics

Eight different antibiotics were used to determine additional selective genetic marker(s) as well as early indicators for the success of conjugation processes (Table 1).

Table (1): Abbreviations and recommended doses of the applied eight antibiotics:

Antibiotic	Abbreviations	Concentration (µg/ml)
Ampicillin	Amp	200
Carbencillin	Carb	100
Streptomycin	Stp	100
Tetracycline	Tet	15
Rifampicin	Rif	150
Kanamycin	Km	100
Chloramphencol	CAMP	100
Cephalexin	Ceph	100

A.4. Pesticides:

Two organophosphorus insecticides; Malathion and Dursban, and one carbamate herbicide; Stomp, were applied with three different doses for each as shown in Table (2).

Table (2): Pesticides under investigation and their applied concentrations.

Pesticide	Dose	Concentration (µl/ml)
Malathion	Zero	0
	1/2 RD	1.25
	RD	2.5
	2RD	5
Dursban	Zero	0
	1/2 RD	2.5
	RD	5
	2RD	10
Stomp	Zero	0
	1/2 RD	3.75
	RD	7.5
	2RD	15

A.5. Reagents and buffers:

Reagents were used for phosphorus determination (Jackson, 1967) and protein analysis buffers (Ogunseitan, 1998).

B. METHODS:

B.1. Phosphate

Isolation of phosphate dissolving bacteria was conducted according to El-Gibaly *et al.*,(1977a). The efficiency of phosphate dissolving bacteria was determined using the methods of Abdel-Hafez (1966); El-Gibaly *et al.*, (1977b) and Abdel-Nasser *et al.*, (1982) while the phosphate dissolving ability was determined using the methods of Jackson (1967).

B.2. Pesticides

To estimate the numbers of pesticides degrading bacteria, plate count agar method was used according to Collins *et al.* (1989) as follows:

B.3. Identification of bacterial isolates:

Cultural, physiological properties and biochemical activities of these isolates were determined as described by Parry *et al.* (1983).On the other hand, *Bacillus* and *Pseudomonas* characterized isolates were more identified according to Bergey's Manual Systematic Bacteriology (1984).

B.4. Conjugation experiments:

Conjugation between bacterial isolates and/or strains was conducted according to the method of Simon (1984).

B.5. Protein analysis:

Extraction of protein was carried out as described by Ogunseitan (1998), while the electrophoresis of proteins was conducted according to the method described by Ried and Collmer (1985).

RESULTS AND DISCUSSION

1. Determination of phosphate dissolving ability:

The ability of selected bacteria to dissolve insoluble phosphate (tricalcium phosphate) to soluble form (calcium mono or diphosphate) was determined quantitatively by measuring soluble phosphorous and pH. The obtained pH and released phosphorus values were collected from media inoculated with the different bacterial isolates after 2, 6, 10 and 14 days during bacterial growth. In addition, the relationship between pH values and the amounts of soluble phosphorus released was studied.

The efficiency of isolates was varied, while some of them were low efficient (less than 100 ppm.of released phosphorus) and the other were high (more than 200 ppm.). The isolate Km15 released the highest amount of phosphorus of 463.33 ppm. The efficiency of the two strains (*B. megaterium* var. *phosphaticum*, *B. megaterium* A.H) was high since they gave 333.33 and 325.33 ppm, respectively at the end of incubation period (14 days).

The data indicated that there were three types of the relationship between pH values and the amounts of soluble phosphorus. The first type included the bacterial isolates which showed remarkable increase in the released phosphorus amounts with the decrease of pH values and was found to contain the majority of the tested bacterial isolates and strains.

The second type included the isolates which increased the pH values with decrease in the amounts of soluble phosphorus.

The third type included the isolates which gave low amounts of soluble phosphorus in spite of their pH values.

The obtained results were in agreement with those obtained by Abdel-Hafez (1966), El-Gibaly *et al.* (1977b) , Abdel-Nasser *et al.* (1982), El-Dahtory *et al.* (1989), Yadav and Singh (1991), Nahas (1996) and Marino *et al.* (1998).

2. Antibiotics resistance and/or sensitivity:

To study the resistance or sensitivity to one or more of eight antibiotics, 40 bacterial isolates and two bacterial strains were chosen according to the variations in their phosphate solublization efficiency. These bacteria were classified into seven classes according to their response to the used antibiotics as shown in Table (3).

3. Pesticides studies:

According to the variations in phosphate dissolving abilities and the response to the used antibiotics, only 20 isolates, in addition to the two strains were selected to study the effect of pesticides on the growth rate. Results in Table (4) present the response of the selected isolates and strains to the different doses of the applied pesticides.

By comparing the number of colonies appeared on the untreated and treated plates, three cases of response to pesticides were obtained. In the first case, very low or no colonies were appeared as a result of application of one or more of the pesticides doses comparing with the untreated controls, these isolates or strains were considered as sensitive (S). In the second case, the colonies appeared on the treated plates were less, in number, than or nearly equal to those appeared on the untreated plates (R). In the third case, some doses caused considerable increase in number of colonies on the treated plates comparing with the controls. The isolates which showed this phenomenon may produce one or more product(s) which cause biodegradation of the pesticide(s) and then decrease or inhibit its effect causing high growth rate of bacterial cells and an increase in colony numbers, these isolates were considered as degradative ones (R*).

Table(3):Categories of genetic markers in the selected bacterial isolates and strains to the used antibiotics.

Class	Isolates and strains	Antibiotics							
		Amp	Carb	Stp	Tet	Rif	Km	CAMP	Ceph.
A	Km10	+	+	+	+	-	-	+	+
	Ks11	+	+	+	+	-	-	+	+
	Kc14	+	+	+	+	-	-	+	+
	Df10	+	+	+	+	-	-	+	+
	Kc15	+	+	+	+	-	-	+	+
	Km1	+	+	+	+	-	-	+	+
	Km2	+	+	+	+	-	-	+	+
	Ks12	+	+	+	+	-	-	+	+
	Gc6	+	+	+	+	-	-	+	+
	Gc11	+	+	+	+	-	-	+	+
	Gs1	+	+	+	+	-	-	+	+
	Gf1	+	+	+	+	-	-	+	+
	Gf2	+	+	+	+	-	-	+	+
	Gf3	+	+	+	+	-	-	+	+
	Gw1	+	+	+	+	-	-	+	+
	Gw6	+	+	+	+	-	-	+	+
	Gw10	+	+	+	+	-	-	+	+
	Dc11	+	+	+	+	-	-	+	+
	Gm15	-	+	+	+	-	+	+	+
B	Gm11	+	+	+	+	-	-	-	+
	Ds10	+	+	+	-	+	-	-	+
	P. putida M.	+	+	-	-	-	+	+	+
C	Ks13	+	-	+	-	+	-	-	+
	Km15	+	-	+	-	+	-	-	+
	Km3	+	+	-	+	-	-	-	+
	Ds4	+	+	+	-	-	-	-	+
	Dw10	+	-	-	+	-	+	-	+
	Kf1	+	-	-	+	+	-	-	+
	Kw1	-	+	+	-	+	+	-	-
	B. megaterium A.H	+	-	+	+	-	-	-	+
D	Km5	+	+	-	-	-	-	-	+
	Dc15	+	+	-	-	-	-	-	+
	Dm1	+	+	-	-	-	-	-	+
	Ds15	+	+	-	-	-	-	-	+
	Dw5	+	+	-	-	-	-	-	+
	Ds1	+	-	-	-	+	-	-	+
	Gs7	+	-	-	-	+	-	-	+
	B. megaterium var. *phosphaticum*	+	+	-	-	-	-	+	-
E	Dc1	+	+	-	-	-	-	-	-
	Km4	+	-	-	-	-	-	-	+
F	Dc13	-	-	-	-	-	-	+	-
	Dm10	-	-	-	-	-	-	-	+
G	Df 7	-	-	-	-	-	-	-	-

+ =:resistant to antibiotics and - = sensitive to antibiotics

Table (4): Final response of the selected isolates and strains to three pesticides.

Isolates and strains	Stomp			Malathion			Dursban		
	1/2 RD	RD	2 RD	1/2 RD	RD	2 RD	1/2 RD	RD	2 RD
Kw1	R*	R*	R*	R*	R*	R*	R*	R*	R*
Dm1	R	R	R	R	R	R	R	R	R
Km15	R*	R*	R*	R*	R*	R*	R*	R*	R*
Kf1	R	R	R	R	R	R	R	R	R
Km10	S	S	S	S	S	S	S	S	S
Ks11	S	S	S	S	S	S	S	S	S
Dc13	S	S	S	S	S	S	S	S	S
Km1	S	S	S	S	S	S	S	S	S
Gw6	S	S	S	S	S	S	S	S	S
Dc1	S	S	S	S	S	S	S	S	S
B. megaterium var. *phosphaticum*	S	S	S	S	S	S	S	S	S
B. megaterium	S	S	S	S	S	S	S	S	S
A.H	R	S	S	R	S	S	R	S	S
Ds 4	R*	R*	R*	R*	R*	R*	S	S	S
Gm11	R*	R*	R*	R*	R*	R*	S	S	S
Df10	R	R	R	R	R	R	S	S	S
Gf1	R	R	R	R	R	R	R	S	S
Gm15	R	R	R	R	R	R	R	S	S
Km2	S	S	S	R	R	R	R	R	R
Gs7	S	S	S	R	R	R	R	R	R
Gf3	S	S	S	S	S	S	R	R	R
Ds1	S	S	S	R	R	R	R	S	S
Gc11									

S=sensitive, R=resistant and R*= degradative ability.

These results were in agreement with those of Spain *et al.* (1980), Spain and van Veld (1983), Hammad *et al.* (1991), Mulbry and Kearney (1991), Gillian (2000) and Digrak and Ozel (2002).

4. Identification of the bacterial isolates:

Morphological, physiological properties and biochemical activities of the 20 selected bacterial isolates were studied according to Parry *et al.* (1983) and Bergey's Manual of Systematic Bacteriology (1984) to classify them.

Comparing the data given in Table (5) with those reported by different investigators (Parry *et al.,* 1983 and Bergey's Manual of Systematic Bacteriology, 1984), it could be concluded that these isolates were classified into two genera ,*Psedomonas* and *bacillus.*

Many studies confirmed that, these two genera had the ability to phosphate solublization,(Patgiri and Bezbaruah ,1990; Mohod *et al.,* 1991; Bilolikar *et al.* , 1996; Gaind and Gaur,1999 and Das *et al.,* 2003). In addition, many authors detected the degradative functions of some *Pseudomonas* and *bacillus* species Matsumura and Boush(1966), Serdar *et al*,(1982), Kao *et al.*(2004) and Neumann *et al.*(2004).

Table (5): Morphological, physiological characteristics and biochemical activities of the 20 selected bacterial isolates.

Isolates	Cell shape	Gram reaction	Motility	Anaerobic growth	Sporulation and spore shape	Pigment production	Oxidase test
Gm11	Short rods	-	Motile	-	-	+	+
Gm15	Short rods	-	Motile	-	-	+	+
km10	Short rods	-	Motile	-	-	+	+
Ks11	Short rods	-	Motile	-	-	+	+
Df10	Short rods	-	Motile	-	-	+	+
Ds1	Short rods	-	Motile	-	-	+	+
Gs7	Short rods	-	Motile	-	-	+	+
Kw1	Short rods	-	Motile	-	-	+	+
Gc11	Short rods	-	Motile	-	-	+	+
Gf1	Short rods	-	Motile	-	-	+	+
Gf3	Short rods	-	Motile	-	-	+	+
Dm1	Short rods	-	Motile	-	-	+	+
Km15	Short rods	-	Motile	-	-	+	+
Km2	Short rods	-	Motile	-	-	+	+
Kf1	Short rods	-	Motile	-	-	+	+
Dc13	Rods	+	Motile	-	+, oval	-	-
Km1	Rods	+	Motile	-	+, oval	-	-
Dc1	Rods	+	Motile	-	+, oval	-	-
Gw6	Rods	+	Motile	-	+, oval	-	-
Ds4	Rods	+	Motile	-	+, oval	-	-

+ = positive and - = negative.

6.A. Conjugation:

Two types of conjugation were used i.e. conjugation between isolates belonging to the same genus of *Pseudomonas* (intrageneric conjugation) and conjugation between strains from the same species of *B. megaterium* (intraspecific conjugation).

To study the intrageneric conjugation, eight *Pseudomonas* isolates (Kf1, Gm15, Km10, Kw1, Ds1, Km15, Gm11 and Gs7) were used. These isolates were proved to be different in their response to more than one antibiotic. Four isolates (Kf1, Gm15, Ds1 and Gs7) exihibited low phosphate dissolving abilities, since they produced 41.66, 43.00, 76.00 and 79.16 ppm of soluble phosphorus, respectively (Table 6). The other isolates showed high phosphate dissolving abilities, since they released more than 300.00 ppm of phosphorus.

All possible intrageneric crosses (eight) between the selected isolates and only one intraspecific cross between two *B. megaterium* strains were carried out as shown in Table (6). For determination of phosphate dissolving efficiency and response to pesticides for the obtained intrageneric and intraspecific transconjugants, the possible nine crosses were classified into three classes according to the phosphate dissolving ability of their parents and each class included three crosses. Fifteen transconjugants were selected randomly from each cross to determine their phosphate dissolving efficiencies. Moreover, from four to eight transconjugants were selected from each cross to test their response to the three used pesticides.

Table (6): Characteristics of the isolates and strains used in intrageneric and intraspecific conjugation crosses.

Class	Crosses No.	Crosses Type	Parents	Phosphate dissolving efficiency (ppm) after 14 days	Response to pesticides: Stomp	Malathion	Dursban	Antibiotic markers
I low x low	1	Intrageneric	Gm15	43.00	R	R	S	Carb^r, Rif^s, CAMP^r
			Kf1	41.66	R	R	R	Carb^s, Rif^r, CAMP^s
	2	Intrageneric	Ds1	76.00	S	S	R	Carb^s, Rif^r, Stp^s
			Gm15	43.00	R	R	S	Carb^r, Rif^s, Stp^r
	3	Intrageneric	Gs 7	79.16	S	R	R	Tet^s, Rif^r, CAMP^s
			Gm15	43.00	R	R	S	Tet^r, Rif^s, CAMP^r
II high x low	4	Intrageneric	Km10	316.60	S	S	S	Carb^r, Rif^s, Stp^r
			Kf1	41.66	R	R	R	Carb^s, Rif^r, Stp^s
	5	Intrageneric	Km15	463.33	R*	R*	R*	Carb^s, Rif^r, Stp^r
			Gm15	43.00	R	R	S	Carb^r, Rif^s, Stp^s
	6	Intrageneric	Gm11	370.00	R*	R*	S	Carb^r, Rif^s, Tet^r
			Ds1	76.00	S	S	R	Carb^s, Rif^r, Tet^s
III high x high	7	Intrageneric	Kw1	316.66	R*	R*	R*	Amp^s, Rif^r, Tet^s
			Km10	316.60	S	S	S	Amp^r, Rif^s, Tet^r
	8	Intrageneric	Km15	463.33	R*	R*	R*	Carb^s, Rif^r, Tet^s
			Km10	316.60	S	S	S	Carb^r, Rif^s, Tet^r
	9	Intraspecific	*B. megaterium* var. *phosphaticum*	333.33	S	S	S	Carb^r, Stp^s, Ceph^s
			B. megaterium A.H	325.33	S	S	S	Carb^s, Stp^r, Ceph^r

a. Class I:

All isolates used as parents in the conjugation in this class exhibited low phosphate dissolving efficiency. Four *Pseudomonas* isolates (Kf1, Gm15, Ds1 and Gs7) were used to carry out the three crosses of this class and all crosses were intrageneric.

a.1. Cross 1:

The results revealed that 14 transconjugants were higher than both parents (Table 7). Four transconjugants (Tr 1/1, Tr 1/5, Tr 1/8 and Tr 1/15) proved to be efficient isolates, since they released phosphorus amounts of 250.00, 233.33, 231.66 and 290.00 ppm., respectively.

On the other hand, the results indicated that all transconjugants except (Tr 1/9 and Tr 1/12), were able to decrease the media pH. This pH decrease was associated with the increase of soluble phosphorus (Table 7).

The obtained results in Table (8) revealed that the two parental isolates, Gm15 and Kf1 were resistant to both stomp and malathion, while they showed an opposite response to dursban.. Three transconjugants, Tr 1/1,Tr 1/5 and Tr 1/8, exhibited resistance to all doses of the three used pesticides. In addition, one transconjugant (Tr 1/15) showed an increase in its colony numbers after treatment with all pesticides doses comparing with control treatment. Therefore, this transconjugant was able to degrade these pesticides and symbolized with R* in Table (8).

Table (7): Amounts of released phosphorus and pH values for the parental isolates of cross 1 and 15 selected transconjugants.

Parents and Transconjugants	pH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Gm15	6.99	6.95	6.92	6.89	33.83	35.33	39.00	43.00	100.00
Kf1	7.56	7.50	7.35	7.30	31.66	33.33	36.66	41.66	96.88
Tr 1/1	5.90	5.88	5.80	5.70	238.33	241.66	246.66	250.00	581.40
Tr 1/2	7.50	7.45	7.39	7.32	31.66	33.16	38.33	40.00	93.02
Tr 1/3	6.95	6.90	6.85	6.90	66.66	75.00	80.00	83.33	193.79
Tr 1/4	7.40	7.00	6.95	6.90	46.66	58.33	71.66	81.66	189.90
Tr 1/5	6.00	5.90	5.87	5.80	163.33	216.66	221.66	233.33	542.62
Tr 1/6	6.50	6.00	5.50	5.20	133.33	141.66	150.00	161.66	375.95
Tr 1/7	7.60	7.40	7.00	6.89	33.33	50.00	62.50	78.33	182.16
Tr 1/8	6.00	5.96	5.87	5.80	164.66	165.83	216.66	231.66	538.74
Tr 1/9	6.90	6.93	6.97	6.99	70.00	75.00	80.00	88.33	205.41
Tr 1/10	7.00	6.90	6.83	6.75	66.66	80.00	91.66	115.00	267.44
Tr 1/11	6.99	6.95	6.90	6.87	73.33	86.66	91.66	100.00	232.55
Tr 1/12	6.87	6.91	6.95	6.98	75.00	81.66	85.00	91.66	213.16
Tr 1/13	7.30	7.10	6.99	7.18	45.00	50.00	48.33	53.33	124.02
Tr 1/14	7.60	7.40	7.20	7.00	41.66	58.33	76.66	81.66	189.90
Tr 1/15	5.94	5.89	5.85	5.75	241.66	250.0	275.00	290.00	674.41

Table(8): Effect of pesticides on number of colonies of high phosphate dissolving transconjugants from cross 1 and their parents.

Parents and trans-conjugants	Control	Pesticides**									Final response**		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
Gm15	140	105	93	70	110	98	75	60	0	0	R	R	S
Kf1	145	110	98	75	115	104	105	125	103	85	R	R	R
Tr 1/1	130	100	75	60	95	75	55	105	90	70	R	R	R
Tr 1/5	100	85	70	55	90	70	50	95	70	60	R	R	R
Tr 1/8	110	80	65	45	100	85	65	88	70	55	R	R	R
Tr 1/15	120	160	200	250	180	240	300	140	200	350	R*	R*	R*

S=sensitive to pesticides, R= resistant to pesticides and R*= degredation.
**St= Stomp, M= Malathion and D= Dursban.

a.2. Cross 2:

Thirteen transconjugants were found to be higher in phosphate dissolving efficiency (Table 9). Five transconjugants (Tr 2/1, Tr 2/2, Tr 2/7, Tr 2/13 and Tr 2/15), out of these 13, showed high efficiency since they exhibited 389.00, 390.33, 358.33, 383.33 and 383.00 ppm, respectively of soluble phosphorus. The other eight transconjugants produced quantities of soluble phosphorus ranged from 100.00 ppm (Tr 2/3) to 183.33 ppm (Tr 2/6).

Results in Table (9) indicated that 12 transconjugants as well as both parents showed inverse correlation between their pH values and soluble phosphorus quantities. On the other hand, three transconjugants (Tr 2/5, Tr

2/8 and Tr 2/14) showed positive correlation. The five transconjugants which showed high phosphate dissolving efficiency in this cross were used for studying their response to the three pesticides under study.

The two parents showed an opposite response to the three pesticides (Table 10). All the five selected transconjugants showed resistance to all pesticides doses with different levels. Two transconjugants (Tr 2/1 and Tr 2/15) were able to resist all doses of the used insecticides. Both transconjugants showed a biodegradation capability to the herbicide! In addition, both transconjugants (Tr 2/7 and Tr 2/13) were able to degrade dursban, but the first transconjugant (Tr 2/7) was able to degrade malathion also. Only one transconjugant (Tr 2/2) resisted all doses of all pesticides.

Table(9):Amounts of released phosphorus and pH values for the parental isolates of cross 2 and 15 selected transconjugants.

Parents and Transconjugants	PH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Ds1	7.05	7.00	6.98	6.95	55.00	67.00	73.00	76.00	100.00
Gm15	6.99	6.95	6.92	6.89	33.83	35.33	39.00	43.00	56.58
Tr 2/1	4.96	4.90	4.81	4.78	350.00	366.66	383.33	389.00	511.84
Tr 2/2	4.89	4.85	4.81	4.78	366.66	385.00	386.00	390.33	513.59
Tr 2/3	6.85	6.83	6.80	6.75	77.50	78.33	83.33	100.00	131.57
Tr 2/4	6.80	6.74	6.70	6.65	100.00	110.00	114.33	120.00	157.89
Tr 2/5	6.90	6.95	6.98	6.98	81.66	91.66	106.66	128.33	168.85
Tr 2/6	5.89	5.86	5.80	5.78	155.00	165.00	180.00	183.33	241.22
Tr 2/7	5.00	4.94	4.90	4.85	316.66	321.66	333.33	358.33	471.49
Tr 2/8	7.00	7.06	7.11	7.19	46.66	53.33	63.33	71.66	94.29
Tr 2/9	7.20	7.10	6.95	6.92	45.00	50.00	58.33	66.33	87.28
Tr 2/10	6.84	6.80	6.75	6.71	83.33	91.66	100.00	108.33	142.54
Tr 2/11	5.93	5.9	5.85	5.81	125.00	145.00	158.00	175.00	230.26
Tr 2/12	5.98	5.93	5.89	5.85	120.00	131.66	136.66	148.30	195.17
Tr 2/13	5.03	5.00	4.95	4.90	333.33	350.00	366.66	383.33	504.38
Tr 2/14	6.20	6.25	6.32	6.39	150.00	161.66	164.66	166.66	219.29
Tr 2/15	4.95	4.90	4.85	4.80	341.66	350.00	366.00	383.00	503.95

Table(10):Effect of pesticides on number of colonies of high phosphate dissolving transconjugants from cross 2 and their parents

Parents and trans-conjugants	Control	Pesticides*									Final response*		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	S	M	D
Ds1	170	0	0	0	0	0	0	108	96	73	S	S	R
Gm15	140	105	93	70	110	98	75	60	0	0	R	R	S
Tr 2/1	100	200	280	400	90	75	60	85	70	55	R*	R	R
Tr 2/2	120	100	85	70	110	95	80	100	80	65	R	R	R
Tr 2/7	140	110	90	75	220	300	400	200	350	450	R	R*	R*
Tr 2/13	100	90	60	45	85	65	50	180	250	400	R	R	R*
Tr 2/15	130	190	360	430	110	90	80	100	75	60	R*	R	R

*St= Stomp, M= Malathion and D= Dursban.

a.3. Cross 3:

Eleven transconjugants exhibited amounts of soluble phosphorus more than their higher parent (Table 11). The highest amounts of soluble phosphorus released by transconjugants Tr 3/3, Tr 3/8, Tr 3/11, Tr 3/14 and Tr 3/15 were 318.66, 333.33, 283.33, 320.33 and 313.66 ppm, respectively. All tested transconjugants were found to be varied in their ability to decrease the media pH values. All selected 15 transconjugants, except Tr 3/9, appeared an inverse relation, as their parents, between pH values and the amounts of released soluble phosphorus (Table 11).

Table(11):Amounts of released phosphorus and pH values for the parental isolates of cross 3 and 15 selected transconjugants.

Parents and Transconjugants	PH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Gs 7	6.90	6.77	6.60	6.50	75.00	76.16	78.33	79.16	100.00
Gm15	6.99	6.95	6.92	6.89	33.83	35.33	39.00	43.00	54.32
Tr 3/1	6.80	6.75	6.71	6.65	83.33	100.00	113.33	126.66	160.00
Tr 3/2	6.74	6.71	6.63	6.60	100.00	110.00	114.33	120.00	151.91
Tr 3/3	4.96	4.90	4.84	4.80	266.33	289.33	309.16	318.66	402.55
Tr 3/4	6.90	6.85	6.80	6.77	71.16	73.33	76.16	78.33	98.95
Tr 3/5	5.89	5.86	5.78	5.75	133.33	155.00	165.00	180.00	227.39
Tr 3/6	7.01	7.00	6.95	6.90	31.83	33.33	35.33	38.66	48.84
Tr 3/7	7.20	7.10	7.05	7.01	28.66	30.00	31.16	32.33	40.84
Tr 3/8	4.89	4.83	4.77	4.69	290.33	313.66	320.00	333.33	421.08
Tr 3/9	6.93	6.98	7.00	7.03	69.33	71.00	72.16	73.33	92.63
Tr 3/10	5.92	5.90	5.84	5.84	125.00	145.00	158.33	175.00	221.07
Tr 3/11	5.30	5.24	5.09	5.02	240.66	251.16	277.00	283.33	357.92
Tr 3/12	5.98	5.95	5.88	5.84	131.66	136.66	148.33	158.33	200.01
Tr 3/13	6.20	6.00	5.90	5.85	133.33	150.00	161.66	166.66	210.53
Tr 3/14	5.05	5.00	4.85	4.78	277.16	285.00	290.00	320.33	404.66
Tr 3/15	4.99	4.95	4.85	4.79	245.00	261.33	279.00	313.66	396.23

Both parental isolates were found to have the same response to malathion, while they showed an opposite response to the other two pesticides (Table 12).

Table(12):Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 3 and their parents.

Parents and trans-conjugants	Control	Pesticides*									Final response*		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	S	M	D
Gs7	200	0	0	0	140	115	85	180	105	95	S	R	R
Gm15	140	105	93	70	110	98	75	60	0	0	R	R	S
Tr 3/3	150	200	280	350	230	330	430	300	370	430	R*	R*	R*
Tr 3/8	120	180	280	380	110	90	80	100	85	75	R*	R	R
Tr 3/11	100	0	0	0	85	70	60	0	0	0	S	R	S
Tr 3/14	200	250	350	450	300	400	450	280	350	460	R*	R*	R*
Tr 3/15	140	0	0	0	120	100	80	0	0	0	S	R	S

*St= Stomp, M= Malathion and D= Dursban.

Two transconjugants (Tr 3/3 and Tr 3/14) were found to be able to degrade all doses of the three used pesticides. Also, it was noticed that one transconjugant (Tr 3/8) showed biodegradation ability to the herbicide stomp only, while it was found to resist the two insecticides. Furthermore, two transconjugants (Tr 3/11) and Tr 3/15) showed similar response to all used pesticides, since they were sensitive to stomp and dursban and resistant to the insecticide, malathion.

b. Class II:

Six isolates were used in the three crosses of this class, three low efficient isolates (Kf1, Gm15 and Ds1) and three highly efficient ones (Km10, Km15 and Gm11). All these isolates showed different responses for some antibiotics and also the pesticides under study.

b.1. Cross 4:

Six transconjugants (Tr 4/1, Tr 4/3, Tr 4/7, Tr 4/9, Tr 4/13 and Tr 4/15) were proved to have the ability to dissolve phosphate more than the higher parent (Table 13). The lowest amounts of released phosphorus were produced by transconjugants Tr 4/8, Tr 4/12 and Tr 4/14. An increase in the amounts of released soluble phosphorus was found to be associated with the decrease of pH for all transconjugants as well as their parents.

Both parental isolates; Km10 and Kf1 showed an opposite response to three pesticides. On the other hand, two transconjugants (Tr 4/1 and Tr 4/15) showed a gradual increase in the total bacterial counts after treating with different doses of stomp and dursban for the first and malathion and dursban for the second (Table 14).

Table(13):Amounts of released phosphorus and pH values for parental isolates of cross 4 and 15 selected transconjugants.

Parents and Trans-conjugants	pH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Km10	5.80	5.70	5.65	5.60	250.80	275.00	300.00	316.60	100.00
Kf1	7.56	7.50	7.35	7.30	31.66	33.33	36.66	41.66	13.16
Tr 4/1	5.43	5.33	5.25	5.20	316.66	333.33	366.66	383.33	121.08
Tr 4/2	6.95	6.90	6.85	6.80	75.00	83.33	100.00	116.66	36.84
Tr 4/3	5.39	5.30	5.26	5.15	335.00	350.00	383.33	400.00	126.34
Tr 4/4	6.50	6.00	5.50	5.40	133.33	150.00	200.00	230.00	72.65
Tr 4/5	5.75	5.70	5.66	5.59	275.00	283.33	298.33	303.33	95.81
Tr 4/6	6.93	6.85	6.80	6.75	70.00	75.00	83.33	108.33	34.22
Tr 4/7	5.57	5.50	5.40	5.32	321.66	325.00	333.33	383.33	121.08
Tr 4/8	7.30	7.10	6.99	7.18	58.33	65.00	73.33	50.00	15.79
Tr 4/9	5.45	5.40	5.36	5.31	375.00	395.00	408.33	416.66	131.60
Tr 4/10	6.99	6.95	6.87	6.82	66.66	75.00	91.66	115.00	36.32
Tr 4/11	5.70	5.63	5.55	5.45	266.66	271.66	283.33	295.00	93.18
Tr 4/12	7.40	7.00	6.95	6.90	46.66	58.33	76.66	83.33	26.32
Tr 4/13	5.65	5.60	5.55	5.45	300.00	310.00	314.33	317.00	100.13
Tr 4/14	7.60	7.40	7.22	7.10	33.33	41.66	50.00	58.33	18.42
Tr 4/15	5.30	5.25	5.15	5.00	331.66	400.00	433.33	450.00	142.13

Table(14):Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 4 and their parents.

Parents and trans-conjugants	Control	Pesticides*									Final response*		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
Km10	220	0	0	0	0	0	0	0	0	0	S	S	S
Kf1	145	110	98	75	115	104	105	125	103	85	R	R	R
Tr 4/1	120	200	280	350	100	85	75	18	300	400	R*	R	R*
Tr 4/3	220	210	150	100	200	120	95	205	130	100	R	R	R
Tr 4/7	200	190	110	90	180	120	85	150	100	75	R	R	R
Tr 4/9	130	110	80	70	120	90	75	190	280	380	R	R	R*
Tr 4/13	140	0	0	0	0	0	0	0	0	0	S	S	S
Tr 4/15	100	90	70	50	150	250	350	200	300	400	R	R*	R*

*St= Stomp, M= Malathion and D= Dursban.

The same phenomenon was observed when transconjugant (Tr 4/9) was treated with all dursban doses compared to the control. Also, two transconjugants (Tr 4/3 and Tr 4/7) were found to be resistant to all pesticides concentrations. Complete lethality was observed for transconjugant (Tr 4/13) when it was treated with all pesticides doses.

2. Cross 5:

Four transconjugants (Tr 5/10, Tr 5/11, Tr 5/12 and Tr 5/15) appeared to have a better ability in releasing phosphorus than their higher parent; Km15 (Table 15). None of the remaining 11 transconjugants produced soluble phosphorus amount less than that amount produced by the lower parent; Gm15, and none of them was considered as low efficient isolate.

The pH values estimated for all the 15 tested transconjugants except Tr 5/4, Tr 5/6 and Tr 5/13 were found to be reduced with an increase in the amounts of soluble phosphorus. In contrast, three transconjugants, Tr 5/4, Tr 5/6 and Tr 5/13 showed an increase in the pH values with the increase in their soluble phosphorus.

Table(15):Amounts of released phosphorus and pH values for the parental isolates of cross 5 and 15 selected transconjugants.

Parents and Transconjugants	pH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Km15	4.84	4.77	4.70	4.64	391.66	441.66	450.00	463.33	100.00
Gm15	6.99	6.95	6.92	6.89	33.83	35.33	39.00	43.00	9.28
Tr 5/1	5.00	4.98	4.95	4.90	333.33	350.00	366.66	383.33	82.73
Tr 5/2	5.05	4.99	4.94	4.89	325.00	331.66	349.83	365.00	78.78
Tr 5/3	5.05	5.00	4.90	4.70	332.50	333.33	366.66	400.00	86.33
Tr 5/4	5.25	5.29	5.33	5.40	330.83	331.66	333.33	358.33	77.33
Tr 5/5	5.30	5.20	5.10	4.95	326.66	333.33	350.00	366.60	79.13
Tr 5/6	4.98	4.99	5.05	5.08	341.66	366.66	385.00	400.00	86.33
Tr 5/7	4.99	4.95	4.90	4.85	350.00	366.66	383.33	400.00	86.33
Tr 5/8	5.15	5.00	4.95	4.90	325.00	333.33	350.00	383.00	82.66
Tr 5/9	5.00	4.96	4.91	4.88	330.00	340.00	360.00	375.00	80.93
Tr 5/10	4.50	4.44	4.35	4.28	466.66	470.00	475.00	483.33	104.32
Tr 5/11	4.50	4.43	4.33	4.29	463.33	475.00	483.00	491.00	105.97
Tr 5/12	4.46	436	4.30	4.20	471.66	481.66	488.33	495.00	106.83
Tr 5/13	5.90	5.95	5.98	5.98	233.33	241.66	243.33	246.66	53.24
Tr 5/14	5.35	5.30	5.25	5.15	335.00	350.00	370.00	390.00	84.17
Tr 5/15	4.40	4.30	4.25	4.20	470.00	475.00	483.33	491.66	106.11

The highest four transconjugants (Tr 5/10, Tr 5/11, Tr 5/12 and Tr 5/15) as well as their parental isolates were used to test their response to the three pesticides. Parental isolate Km15, was found to be able to degrade all doses of these pesticides while the other parental isolate (Gm15) was resistant to both stomp and malathion, but it was sensitive to the insecticide, dursban (Table 16). All of the selected transconjugants were found to have a biodegradation capability towards the two pesticides stomp and malathion. Two transconjugants (Tr 5/10 and Tr 5/15) were found to have the ability to degrade dursban, while transconjugants (Tr 5/11and Tr 5/12) were resistant only to dursban.

Table(16):Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 5 and their parents.

Parents and trans-conjugants	Control	Pesticides*									Final response*		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
Km15	215	285	350	435	310	370	460	310	380	485	R*	R*	R*
Gm15	140	105	93	70	110	98	75	60	0	0	R	R	S
Tr 5/10	150	200	280	350	250	350	450	180	280	380	R*	R*	R*
Tr 5/11	200	250	370	430	260	380	460	180	150	100	R*	R*	R
Tr 5/12	220	280	380	460	300	400	500	200	120	100	R*	R*	R
Tr 5/15	160	240	300	420	200	320	440	220	350	450	R*	R*	R*

*St= Stomp, M= Malathion and D= Dursban.

b.3.Cross 6:

It was noticed that four transconjugants (Tr 6/1, Tr 6/6, Tr 6/10 and Tr 6/13) manifested superiority in releasing phosphorus over the higher parental isolate; Gm11, while they produced 383.33, 400.00, 385.00 and 408.33 ppm of phosphorus, respectively. In contrast, the other four transconjugants (Tr 6/2, Tr 6/3, Tr 6/4 and Tr 6/14) exhibited amounts of released phosphorus lower than that of the lower parental parent, Ds1.

Table(17):Amounts of released phosphorus and pH values for the parental isolates of cross 6 and 15 selected transconjugants.

Parents and Transconjugants	pH				Soluble-P (ppm)				% from Higher Parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Gm11	5.20	4.94	4.88	4.80	330.00	331.60	336.60	370.00	100.00
Ds1	7.05	7.00	6.98	6.95	55.00	67.00	73.00	76.00	20.54
Tr 6/1	5.57	5.50	5.40	5.30	321.66	325.00	333.33	383.33	103.60
Tr 6/2	7.30	7.25	7.17	7.10	35.00	41.66	46.66	48.33	13.06
Tr 6/3	6.99	7.06	7.11	7.18	28.33	33.33	50.00	58.33	15.76
Tr 6/4	6.90	6.85	6.80	7.00	40.00	48.33	55.00	38.33	10.36
Tr 6/5	5.70	5.63	5.55	5.45	266.66	272.00	284.00	295.00	79.72
Tr 6/6	5.39	5.30	5.25	5.15	332.00	347.00	380.00	400.00	108.10
Tr 6/7	5.38	5.35	5.30	5.25	321.66	328.33	348.33	358.33	96.84
Tr 6/8	5.93	5.89	5.93	6.02	125.00	145.00	128.33	115.00	31.08
Tr 6/9	5.30	5.20	5.15	5.05	326.66	331.66	333.33	350.00	94.59
Tr 6/10	5.43	5.33	5.25	5.20	317.00	334.00	367.00	385.00	104.05
Tr 6/11	5.89	5.82	5.78	5.75	133.33	155.00	165.00	180.00	48.65
Tr 6/12	6.00	5.90	5.85	5.80	165.00	216.66	225.00	233.33	63.06
Tr 6/13	5.30	5.25	5.16	5.04	383.33	391.66	400.00	408.33	110.36
Tr 6/14	7.05	7.12	7.15	7.22	37.50	50.00	60.00	68.00	18.38
Tr 6/15	7.00	6.95	6.90	6.85	50.00	56.66	63.33	76.00	20.54

One transconjugant, (Tr 6/15) released an amount of soluble phosphorus equal to that produced by the lower parental isolate; Ds1 (76.00 ppm). The remaining six transconjugants showed intermediate efficiency.

The pH values estimated during the incubation period indicated that 11 transconjugants as well as their parents had the ability to bring down the pH of the media. The amounts of released phosphorus associated with this reduction were increased (Table 17). In addition, two transconjugants (Tr 6/3 and Tr 6/14) showed an increase in the pH value of the media with a corresponding increase in the amounts of released phosphorus.

To study whether the transconjugants can grow or not as a result of the pesticide treatments, four transconjugants (Tr 6/1, Tr 6/6, Tr 6/10 and Tr 6/13) which produced soluble phosphorus amounts more than the higher parent were selected as shown in Table (18).

Table(18):Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 6 and their parents.

Parents and transconjugants	Control	Pesticides									Final response		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
Gm11	190	210	420	498	220	440	518	0	0	0	R*	R*	S
Ds1	170	0	0	0	0	0	0	108	96	73	S	S	R
Tr 6/1	200	250	330	450	270	380	470	190	110	80	R*	R*	R
Tr 6/6	160	200	340	440	190	250	380	140	100	75	R*	R*	R
Tr 6/10	220	250	350	450	260	380	470	200	150	100	R*	R*	R
Tr 6/13	150	200	300	400	220	350	460	180	240	380	R*	R*	R*

***St= Stomp, M= Malathion and D= Dursban.**

The results obtained in Table (18) showed that the two parental isolates varied in their response to the three applied pesticides, while all the tested transconjugants degraded stomp and malathion. Also, the same finding was observed when transconjugant Tr 6/13 was treated with all doses of dursban. Three transconjugants, Tr 6/1, Tr 6/6 and Tr 6/10 were found to be resistant only to dursban doses.

c. Class III:

Two intrageneric crosses (7 and 8) between three *Pseudomonas* isolates (Km15, Km10 and Kw1) were applied. Also, one intraspecific cross (No. 9) was carried out between two *B. megaterium* strains (*B. megaterium* var. *phosphaticum* and *B. megaterium* A.H). All bacterial isolates and strains used in this class showed highly phosphate dissolving abilities.

c.1. Cross 7:

All the selected transconjugants showed higher capacity of dissolving tricalcium phosphate than their parents (Table 19). The highest production of released phosphorus was obtained by transconjugant; Tr 7/10 (433.33 ppm) and the lowest amount (366.33 ppm) was produced by the transconjugant (Tr 7/9) which was also higher than its parents.

Table(19):Amounts of released phosphorus and pH values for the parental isolates of cross 7 and 15 selected transconjugants.

Parents and Transconjucants	PH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Kw1	5.85	5.76	5.69	5.60	275.83	285.00	303.33	316.66	100.00
Km10	5.80	5.70	5.65	5.60	250.80	275.00	300.00	316.60	99.98
Tr 7/1	5.35	5.30	5.25	5.20	325.83	350.00	383.33	400.00	126.32
Tr 7/2	5.60	5.50	5.45	5.35	316.66	330.83	350.00	366.66	115.79
Tr 7/3	5.40	5.33	5.28	5.22	330.00	358.33	383.33	408.33	128.95
Tr 7/4	5.55	5.66	5.70	5.81	229.16	331.66	363.33	381.66	120.53
Tr 7/5	4.95	4.90	4.85	4.76	333.33	366.66	383.33	400.00	126.32
Tr 7/6	5.10	4.97	4.90	4.85	350.00	380.00	400.00	416.66	131.58
Tr 7/7	5.24	5.20	5.00	4.86	373.33	398.33	410.00	428.33	135.26
Tr 7/8	5.38	5.30	5.25	5.20	326.66	330.83	366.66	383.33	121.05
Tr 7/9	5.35	5.30	5.20	5.08	330.00	333.33	349.83	366.33	115.69
Tr 7/10	4.98	4.93	4.89	4.80	375.00	400.00	416.66	433.33	136.84
Tr 7/11	5.26	5.18	5.00	4.88	363.33	376.66	400.00	416.66	131.58
Tr 7/12	4.99	4.90	4.85	4.80	341.66	366.66	383.33	400.00	126.32
Tr 7/13	5.13	4.96	4.92	4.88	376.66	393.33	411.66	426.66	134.74
Tr 7/14	5.00	4.97	4.91	4.78	380.00	395.00	410.00	430.00	135.79
Tr 7/15	4.98	4.92	4.89	4.83	358.33	381.66	398.33	415.00	131.05

The results clearly showed that the increase in the soluble phosphorus amounts was associated with the reduction of pH values of the media. In addition, this increase in the phosphorus amounts was noticed also with the transconjugant Tr 7/4 which increased the pH of the media.

For testing the effects of the used pesticides on transconjugants survival, eight transconjugants which released more than 400.00 ppm were selected as shown in Table (20).

The parental isolate Km10 was found to be sensitive to all the three pesticides doses, while the other parent Kw 1 was found to be successful in degrading them.

Six transconjugants (Tr 7/6, Tr 7/7, Tr 7/10, Tr 7/13, Tr 7/14 and Tr 7/15) exhibited a response similar to that of the parental isolate Kw1. On contrast, the three pesticides were more resistant to biodegradation by the other two tested transconjugants (Tr 7/3 and Tr 7/11) while their growth was inhibited after exposure to all doses of pesticides.

c.2. Cross 8:

The second cross of this class was achieved between the two highly efficient isolates in phosphate dissolving (Km15 and Km10).The seven transconjugants; Tr 8/1, Tr 8/4, Tr 8/7, Tr 8/8, Tr 8/12, Tr 8/13 and Tr 8/15 showed the highest activity in phosphate solubilization and were higher in their productivity than the higher parent, since they gave more than 500.00, 470.00, 483.33, more than 500.00, 491.66, 485.00 and more than 500.00 ppm of released phosphorus, respectively (Table 21). In addition, seven transconjugants showed intermediate productivity and only one transconjugant (Tr 8/14) was equal to the lower parent in its productivity.

Table (20): Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 7 and their parents

Parents and trans-conjugants	Control	Pesticides*									Final response*		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
Kv 1	220	290	345	430	310	365	450	300	420	550	R*	R*	R*
Km 10	220	0	0	0	0	0	0	0	0	0	S	S	S
Tr 7/3	200	0	0	0	0	0	0	0	0	0	S	S	S
Tr 7/6	150	200	280	380	190	250	400	210	320	430	R*	R*	R*
Tr 7/7	230	270	380	460	250	370	480	280	380	500	R*	R*	R*
Tr 7/10	140	180	260	370	190	290	390	200	300	400	R*	R*	R*
Tr 7/11	150	0	0	0	0	0	0	0	0	0	S	S	S
Tr 7/13	180	230	350	460	220	370	480	250	360	480	R*	R*	R*
Tr 7/14	220	270	380	450	250	360	480	300	420	530	R*	R*	R*
Tr 7/15	200	260	370	480	300	420	540	280	380	450	R*	R*	R*

*St= Stomp, M= Malathion and D= Dursban.

Table(21):Amounts of released phosphorus and pH values for the parental isolates of cross 8 and 15 selected transconjugants.

Parents and transconjugants	pH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
Km15	4.84	4.77	4.70	4.64	391.66	441.66	450.00	463.33	100.00
Km10	5.80	5.70	5.65	5.60	250.80	275.00	300.00	316.60	68.33
Tr 8/1	4.16	4.10	4.05	3.99	482.16	491.66	500.00	>500.00	107.91
Tr 8/2	5.60	5.55	5.50	5.45	308.33	316.66	325.00	333.33	71.94
Tr 8/3	5.53	5.48	5.40	5.35	321.66	330.00	338.33	358.33	77.34
Tr 8/4	5.25	5.20	5.15	5.10	438.33	448.33	461.66	470.00	101.44
Tr 8/5	5.20	5.17	5.12	5.07	425.00	433.33	441.66	450.00	97.12
Tr 8/6	4.95	4.89	4.85	4.80	433.00	441.00	450.00	458.00	98.85
Tr 8/7	4.55	4.50	4.45	4.40	455.00	466.66	475.00	483.33	104.32
Tr 8/8	4.50	4.44	4.34	4.30	478.33	483.33	490.00	>500.00	107.91
Tr 8/9	5.60	5.53	5.49	5.44	320.00	325.00	331.66	333.33	7[illegible].94
Tr 8/10	5.40	5.35	5.30	5.25	383.33	408.33	425.00	438.30	94.60
Tr 8/11	5.25	5.20	5.15	5.05	358.33	378.00	400.00	415.00	89.57
Tr 8/12	4.95	4.90	4.85	4.80	466.33	476.66	483.33	491.66	106.11
Tr 8/13	5.00	4.88	4.84	4.80	450.00	466.50	475.00	485.00	104.68
Tr 8/14	5.90	5.85	5.80	5.75	255.00	276.66	297.83	316.60	68.33
Tr 8/15	4.30	4.25	4.15	4.05	490.00	495.00	500.00	>500.00	107.91

The amounts of soluble phosphorus released by all tested transconjugants showed an inverse relation with the pH values (Table 21). All transconjugants obtained from this cross were able to bring down the pH in the acidity direction which was associated with an increase in the quantity of soluble phosphorus released.

To study the effects of each of the three used pesticides on the grown transconjugants, only seven transconjugants were selected, since they were more efficient than the highest parent (Km15).

Data presented in Table (22) revealed that both parents showed an opposite response to pesticides. Also, five transconjugants (Tr 8/1, Tr 8/4, Tr 8/8, Tr 8/12 and Tr 8/15 had a biodegradation ability to all used pesticides. In addition their total colony counts were found to be higher than the control.

Two transconjugants (Tr 8/7 and Tr 8/13) showed a degree of resistance less than the other five, both transconjugants were considered as resistant ones for all doses of pesticides.

Table (22): Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 8 and their parents.

Parents and trans-conjugants	Control	Pesticides*									Final response*		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
Km 15	215	285	350	435	310	370	460	310	380	485	R*	R*	R*
Km 10	220	0	0	0	0	0	0	0	0	0	S	S	S
Tr 8/1	200	250	350	450	260	370	480	300	400	500	R*	R*	R*
Tr 8/4	180	240	320	400	250	380	480	200	350	450	R*	R*	R*
Tr 8/7	250	230	180	120	220	170	100	240	190	120	R	R	R
Tr 8/8	140	180	250	370	200	320	430	190	250	360	R*	R*	R*
Tr 8/12	150	200	300	400	220	330	440	180	250	370	R*	R*	R*
Tr 8/13	220	210	150	100	200	140	100	205	170	100	R	R	R
Tr 8/15	160	220	330	450	240	360	480	250	350	450	R*	R*	R*

*St= Stomp, M= Malathion and D= Dursban.

3. Cross 9:

The third cross of this class was intraspecific and carried out between two *B. megaterium* strains, *B. megaterium* var. *phosphaticum*, which is used as a commercial inoculom, and *B. megaterium* A.H. (Table 23).

All tested transconjugants showed variable levels of phosphate solubilization. The production of soluble phosphorus of 14 transconjugants was found to be higher than that released by the higher parental strain; *B. megaterium* var. *phosphaticum*. The soluble phosphorus productivity of these transconjugants ranged from 337.00 ppm (Tr 9/3) to more than 500.00 ppm (Tr 9/15). Only one transconjugant (Tr 9/2) released a quantity of phosphorus (333.33 ppm) equal to that released by the highest parental strain; *B. megaterium* var. *phosphaticum* (Table 23).

Eleven transconjugants were found to exhibit an inverse relation between the amounts of soluble phosphorus and their pH values (Table 23). The increase in the quantities of soluble phosphorus released by these transconjugants was associated with reduction in pH values of their media. In addition, two transconjugants (Tr 9/1 and Tr 9/5) showed a positive relationship between pH values and the amounts of soluble phosphorus, since the increase in pH values was accompanied by an increase in the amounts of soluble phosphorus. Another type of relationship was noticed in the case of the transconjugant (Tr 9/2), while an increase in the pH values

Table (23): Amounts of released phosphorus and pH values for the parental isolates of cross 9 and 15 selected transconjugants.

Parents and transconjugants	pH				Soluble-P (ppm)				% from higher parent
	Days after inoculation				Days after inoculation				
	2	6	10	14	2	6	10	14	
B. megaterium var. *phosphaticum*	5.45	5.25	5.15	4.99	328.33	331.66	332.83	333.33	100.00
B. megaterium A.H	5.70	5.50	5.40	5.30	317.33	320.66	322.83	325.33	97.60
Tr 9/1	5.35	5.42	5.50	5.55	330.00	333.00	338.00	340.00	102.00
Tr 9/2	5.45	5.48	5.56	5.60	345.33	339.00	333.33	333.33	100.00
Tr 9/3	5.45	5.40	5.35	5.20	323.00	328.00	334.00	337.00	101.10
Tr 9/4	5.18	5.05	4.90	4.85	339.00	345.00	350.00	362.00	108.60
Tr 9/5	5.20	5.21	5.28	5.35	330.00	334.00	338.00	351.00	105.30
Tr 9/6	5.10	5.00	4.90	4.86	340.00	350.00	360.00	365.00	109.50
Tr 9/7	4.90	4.80	4.70	4.65	346.00	358.00	369.00	430.00	129.00
Tr 9/8	4.95	4.90	4.85	4.80	340.00	345.00	350.00	400.00	120.00
Tr 9/9	4.80	4.72	4.64	4.58	353.00	367.00	375.00	420.00	126.00
Tr 9/10	4.40	4.30	4.25	4.20	475.00	478.00	483.33	491.66	147.49
Tr 9/11	4.30	4.20	4.15	4.10	480.83	483.33	491.66	498.33	149.50
Tr 9/12	5.27	5.28	5.29	5.29	327.00	336.00	341.00	350.00	105.00
Tr 9/13	4.41	4.36	4.30	4.20	481.66	488.33	495.00	499.16	149.75
Tr 9/14	4.33	4.22	4.18	4.05	491.66	495.83	498.33	500.00	150.00
Tr 9/15	4.30	4.27	4.08	4.00	479.16	483.33	499.50	> 500.00	> 150.00

Growth inhibition was noticed when both parental strains were treated with all concentrations of pesticides(Table 24). Eight highly efficient transconjugants (Tr 9/7, Tr 9/8, Tr 9/9, Tr 9/10, Tr 9/11, Tr 9/13, Tr 9/14 and Tr 9/15) were selected to study the effect of the used pesticides.

Table (24): Effect of pesticides on number of colonies of the high phosphate dissolving transconjugants from cross 9 and their parents.

Parents and Trans-conjugants	Control	Pesticides									Final response		
		St			M			D					
		½ RD	RD	2RD	½ RD	RD	2RD	½ RD	RD	2RD	St	M	D
B.megaterium var. *phosphaticum*	205	0	0	0	0	0	0	0	0	0	S	S	S
B.megaterium A.H	175	0	0	0	0	0	0	0	0	0	S	S	S
Tr 9/7	200	0	0	0	0	0	0	0	0	0	S	S	S
Tr 9/8	180	0	0	0	0	0	0	0	0	0	S	S	S
Tr 9/9	150	0	0	0	0	0	0	0	0	0	S	S	S
Tr 9/10	220	0	0	0	0	0	0	0	0	0	S	S	S
Tr 9/11	160	0	0	0	150	100	80	140	90	75	S	R	R
Tr 9/13	240	220	150	100	230	170	120	200	120	70	R	R	R
Tr 9/14	160	0	0	0	0	0	0	0	0	0	S	S	S
Tr 9/15	230	220	160	100	0	0	0	210	180	120	R	S	R

*St= Stomp, M= Malathion and D= Dursban.

While only one transconjugant (Tr 9/13) was found to exhibit complete resistance to all pesticides doses, two transconjugants (Tr 9/11 and

Tr 9/15) exhibited resistance to two pesticides.On the other hand, five transconjugants (Tr 9/7, Tr 9/8, Tr 9/9, Tr 9/10 and Tr 9/14) were unable to resist any of the applied pesticides doses.

Concerning the results shown in Tables (from 7 to 24), it can be concluded that 16 from 135 selected transconjugants proved to have the ability to release phosphorus amounts more than the highest parental isolate used in this study (Km15). Out of these 16 transconjugants, four (Tr 5/10, Tr 5/11, Tr 5/12 and Tr 5/15) were belonging to cross 5 in class II. In addition, these transconjugants had the ability to degrade both stomp and malathion. Also, only two transconjugations (Tr 5/10 and Tr 5/15) degraded dursban, but the other two (Tr 5/11 and Tr 5/12) were resistant.

On the other hand, seven high phosphate producing transconjugants (Tr 8/1, Tr 8/4, Tr 8/7, Tr 8/8, Tr 8/12, Tr 8/13 and Tr 8/15) were obtained from cross 8 in class III. Five out of these seven transconjugants (Tr 8/1, Tr 8/4, Tr 8/8, Tr 8/12 and Tr 8/15) had the ability to degrade all pesticides while two transconjugants (Tr 8/7 and Tr 8/13) were resistant for all pesticides.

Also, five high phosphate dissolving bacterial transconjugants (Tr 9/10, Tr 9/11, Tr 9/13, Tr 9/14 and Tr 9/15) were obtained from the intraspecific cross (cross 9). Two transconjugants (Tr 9/10 and Tr 9/14) were found to be sensitive for all pesticides doses and one transconjugant (Tr 9/13) showed high resistance for pesticides doses. On the other hand, transconjugant (Tr 9/11) was resistant to malathion and dursban and sensitive to stomp while (Tr 9/15) was sensitive to malathion and resistant to the other two pesticides.

It was noticed that, both crosses (5 and 8) which gave 11 high phosphate dissolving transconjugants were including the highest parent (Km15). Moreover, isolate Km15 shared only in these crosses as a parent, but did not use in other crosses in the conjugation experiments. It could be concluded that, when isolate Km15 was used with either low efficient parent (Gm15 in cross 5) or highly efficient parent (Km10 in cross 8), it proved to have the ability to inherit the high phosphate dissolving efficiency to many of its transconjugants. This means that this isolate carries the gene(s) which could be responsible for high phosphate dissolving efficiency. Moreover, the majority of the transconjugants selected from the two crosses (5 and 8) proved to have the ability to degrade all doses of pesticides with some minor exceptions. This may indicate that isolate Km15 also contains the degradative gene(s), which could be responsible for the biodegradation of these pesticides. To determine if both types of genes are carrying on either the chromosomal DNA, the plasmid DNA or distributed between both DNAs, further studies will be needed.

On the other hand, it was not surprising that many transconjugants from cross 9 showed the ability to produce highly phosphate amounts, while both parents (*B. megaterium* strains) are well known as high phosphate producers. In other words, they may harbor the gene(s) responsible for dissolving phosphate. But, none of these transconjugants showed any pesticides degradative ability as shown in Table (24), this indicate that both strains may not carry the degradative gene(s), which are responsible for biodegradation process of pesticides.

By studying DNA transfer results which were obtained after the application of conjugation experiments in this work, it could be concluded that these results took three main phenomena into consideration. These main phenomena were the efficiency of releasing soluble phosphorus, the relationship between pH values and the amounts of released phosphorus and the response to three applied pesticides. These subjects were studied in all selected transconjugants and compared with the results of the parents.

For the first phenomenon; the highly efficiency of releasing soluble phosphorus: Higher phosphate productivity may be a result of reactivation of some genes which are responsible for this phenomenon as a result of collecting different genetic materials in one cell as a result of conjugation. Also, this gene activation may be a result of the effect of some cytoplasmic components which were transferred from one cell to another.
Another interpretation for the higher productivity of some of the obtained recombinants in comparison with their parents, that the genes which are responsible for phosphate dissolving were suppressed in its own host cells (one or both parents) and once these genes were transferred or collected with other genetic components, they could be reactivated and share with other genes in the enhancement of phosphate dissolving. These results are in agreement with those of Goldstein (1995), Rodriguez *et al.* (2001), Zhao *et al.* (2002) and Goldstein *et al.*, (2003).

For the second phenomenon; the relationship between pH values and the amounts of released phosphorus for the obtained transconjugants: Two types of relations were found. The first was reverse association between pH values and amounts of released phosphorus. The second was positive association; this means that the increase of phosphorus amounts with the increase of pH values.

It is well known that low pH value is an essential factor for the conversion of insoluble form of phosphorus to the soluble one. Phosphate dissolving bacteria have the ability to produce organic acids such as gluconic acid and 2-ketogluconic acid from glucose. Results of this study indicated that the new constructed genetic pools found as a result of mixing the genetic materials of two cells (conjugation) may activate some gene(s) in glucose pathway, which encourage the induction of these organic acids, causing the decrease of pH values and then enhancing the release of soluble phosphorus. These results are in agreement with those of Marino *et al.* (1998), Gaind and Gaur (1999) and Xu *et al.* (2004).

For the third phenomenon; the ability of some bacterial isolates on resistance or degrading different pesticides:

Results of this study revealed that some of the transconjugants were sensitive to one or more of the three pesticides used in this study. These transconjugants may lack the gene(s) responsible for the resistance to pesticide(s) or these recombinants may carry the resistance gene(s) but in inactive form, therefore, these recombinants seem as sensitive isolates for this pesticide(s). Some other transconjugants had the ability to resist the effect of one or more of pesticides, while they grew on media containing this pesticide(s). These recombinants may carry one or more of the detoxification genes which prevent the toxic effect of pesticide(s). This prevention can occur by cleavage of one or more of the toxic groups or disruption of chemical bond(s) which are important for the chemical structure of pesticide(s). Also, pesticides resistance gene(s) may be carried by these new constructed isolates and prevent the penetration of pesticide(s) into the bacterial cell or indicate this pesticide(s) once it penetrate into the cell. The occurrence of one of these effects is dependent on the location of this gene(s) in the new constructed cell, if it is carried on either the chromosomal or plasmid DNA.

Other transconjugants showed the ability of pesticides biodegradation and they may carry one or more of the degradative genes. This gene(s) have the ability to degrade or breakdown the pesticide(s) to its precursors or primary substrates. These substances can be utilized by the new transconjugants and enhance their growth and give large numbers of bacterial cells on the pesticide-treated plates in comparison with the controls. These results are in agreement with many authors who used DNA transfer techniques to improve the pesticide biodegradation ability. Suwa *et al.* (1996), Feng and Kennedy (1997), Paromenskaya *et al.* (1998), Hayatsu *et al.* (1999), Sutherland *et al.* (2002) and Desaint *et al.* (2003).

For the transconjugant Tr 6/13 (Tables 21 and 39) which showed degradative ability for the insecticide dursban, in spite of one of its parents (Gm11) was sensitive and the second (Ds1) was resistant for the same insecticide, this result agree with that reported by Kole and Dey (1989). They found that when some pesticides such as Stomp and Ronstar were used at or above recommended rates, the growth of phosphate dissolving bacteria was inhibited. They added that the effect was more pronounced at higher rates of the herbicides and decreased with time after application due to herbicide biodegradation. Finally, it can be concluded that the application of DNA transfer technique such as conjugation led to the construction of different types of recombinants with DNA molecules from different sources. The majority of these recombinants showed higher abilities in solubilizing phosphate and resisting or degrading pesticides.

7. Genetic analysis of protein patterns:

To identify a biochemical genetic marker associated with phosphate solubilization trait, SDS-PAGE technique was used. Twelve transconjugants out of the 15 resulted from cross 4 (Km10 x Kf1) differing in their productivity of soluble phosphorus in addition to their parents were chosen. Out of the 12 selected transconjugants, eight had high phosphate solubilization efficiency, (Tr 4/15, Tr 4/9, Tr 4/3, Tr 4/1, Tr 4/13, Tr 4/5, Tr 4/11 and Tr 4/4) and four showed low phosphate dissolving efficiency (Tr 4/6, Tr 4/12, Tr 4/14 and Tr 4/8) were used. Electrophoresis analysis for the extracted proteins of the 12 selected transconjugants and their parents was carried out. Figure (1) presents the banding patterns and the molecular weight of the extracted proteins against a standard protein marker (Cat. N. 161.0309, BioRAD).

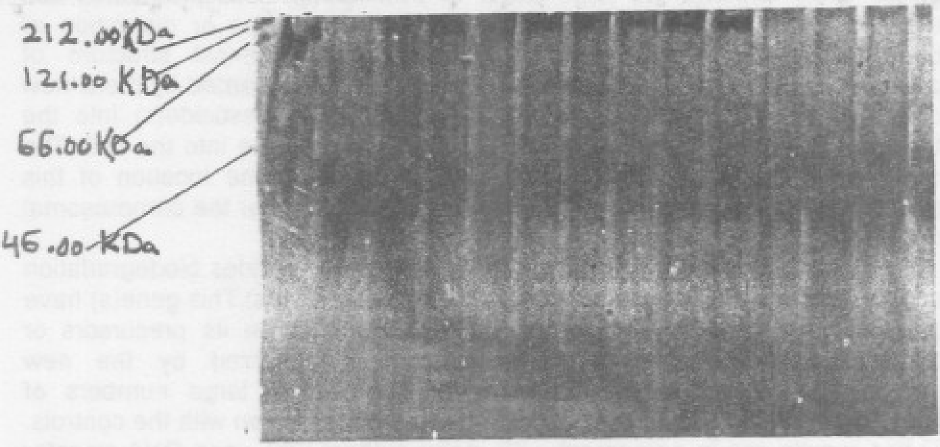

Figure (1): A photograph representing SDS-PAGE banding patterns of *Pseudomonas* isolates, and 12 of their transconjugants.

There were remarkable variations in the numbers of protein bands between the two parental isolates, while eight bands were detected in SDS-PAGE banding patterns for the highly efficient isolate (Km10) and only four bands for the low efficient isolate (Kf1). The highest band numbers were detected in the first four highly efficient transconjugants; Tr 4/15, Tr 4/9, Tr 4/3 and Tr 4/1, the intensity of these bands was differed. On the other hand, the other four highly efficient transconjugants showed different protein band numbers, while ten identical bands were detected for both Tr 4/13 and Tr 4/5, eight and nine bands were detected for Tr 4/11 and Tr 4/4, respectively.
With regard to the rest four low efficient transconjugants, it was noticed that seven identical bands were detected for both transconjugants; Tr 4/6 and Tr 4/8 and nine identical bands were detected for both transconjugants; Tr 4/12 and Tr 4/14.

The results revealed also some differences in the intensity of the same protein bands between the parental isolates and their transconjugants. The high efficient parent; Km10 represented a weak intensity band (No. 4), which was very weak in the high efficient eight transconjugants. This band was absent in the low efficient parent (Kf1) and the four low efficient transconjugants. In addition, Km10 isolate exhibited very weak intensity bands (Nos. 10, 11 and 12), which were detected as weak bands only in the first four of the high and all the low efficient tested transconjugants.

These results confirmed those obtained by Tegelström and Essen (1996) who reported that individuals originating from different breeders are more dissimilar than those from the same breeder. El-Sanossy (2003), indicated that there were levels of polymorphism among some *Azotobacter* strains and their resulted transconjugants. He also showed that the intensity of protein bands was differed at the same band from the parental strains to their resulted transconjugants.

This study showed that one protein band (No. 2) with size of 66 KDa may be associated with phosphate solubilizing activity, while it was detected in the high efficient parent; Km10 and eight high efficient transconjugants.

It can be concluded that protein analysis may be used as biochemical genetic tool to identify bacterial isolates and their recombinants with specific characteristics. However, further studies on other molecular markers such as RFLP and RAPD are needed to substantiate this conclusion.

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إنتاج عزلات بكترية عالية الكفاءة في إذابة الفوسفات والهدم الحيوي للمبيدات من خلال التزاوج البكتيري النوعي والبين نوعي

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أجرى هذا البحث بهدف الحصول على عزلات بكتيرية ذات كفاءة عالية في اذابة الفوسفات ومقاومة فعل أوالهدم الحيقي للمبيدات وذلك عن طريق التزاوج البكتيرى. ولتحقيق ذلك تم استخدام ٢٠٠ عزلة بكتيرية تم عزلها من منطقة الرزوسفير لمحاصيل مختلفة هى القطن،المانج بين، فول الصويا ، الفول البلدى والقمح وذلك من ثلاثة محافظات مختلفة هى كفرالشيخ والغربية و الدقهلية من اراضي سبق معاملتها بالمبيدات هذا بالاضافة الى سلالتين تابعتين لجنس الباسيلس أحداهما تستخدم كلقاح بكتيرى على نطاق تجارى. وقد تم اجراء البحث على ستة مراحل كالتالى.

١- تم تحديد كفاءة جميع العزلات فى اذابة الفوسفات حيث كانت هناك اختلافات معنوية بين العزلات فى كفاءتها وكانت اعلى العزلات كفاءة هى (Km 15) والتى اعطت ٣٣ر٤٦٣جزء فى المليون.
٢- أظهرت ٤٠ عزلة بكتيرية تم انتخابها علىاساس اختلاف قدرتها فىاذابة الفوسفات بالاضافة الى سلالتى الباسيلس مستويات مختلفة من حيث مقاومتها أو حساسيتها لثمانية من المضادات الحيوية .
٣- أظهرت ٢٠ عزلة بكتيرية تم انتخابها على أساس اختلاف استجابتها للمضادات الحيوية اختلافات فى مدى قدرتها على مقاومة أو هدم أثنين من المبيدات الحشرية بالاضافة الى مبيد حشائش واحد.
٤- باستخدام بعض الأختبارات المورفولوجية والفسيولوجية و البيوكيميائية وجد أن هذة العزلات تتبع أحد الجنسين البكتيريين الباسيلس أو السيدوموناس.
٥- قسمت تجارب التزاوج الجنسى الى ثلاثة أقسام تضمن كل جزء منها ثلاث تهجينات ثم تم اختيار١٥ متحولة وراثية (Transconjucants) من كل هجين لدراسة كفاءتها في اذابة الفوسفات مقارنة بالأباء المستخدمة ثم تم اختيار ما بين ٤ الى ٨ Transconjugants من تلك التى أظهرت كفاءة عالية فى عملية الاذابة لدراسة تأثير المبيدات المستخدمة عليها.

أوضحت النتائج أن من بين ال ١٣٥ Transconjugants كان هناك ١٦ ذات قدرة عالية فى اذابة الفوسفات مقارنة باعلى الأباء المستخدمة (Km15) كما كان لمعظمها القدرة على الهدم الحيوي لجميع المبيدات المستخدمة.

٦- أظهر التحليل البيوكيميائى للبروتين الذى اجرى على ١٢ متحولة وراثية Transconjugants ناتجة من الاقتران الجنسى بين العزلتين Km10 و Kf1 فى محاولة لايجاد كشاف بيوكيميائى لصفة اذابة الفوسفات أن هناك حزمة واحدة ذات حجم ٦٦ كيلو دالتون تميز الهجن ذات الكفائة العالية.