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Transformation of Agrobacterium Tumefactions by Conjugation Technique

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Abstract: Two genera of Rhizobiaceae ,wild strain Agrobacterium tumefaciens (AtMcol) and standerd strain Agrobacterium tumefaciens (C58C1), in addition to Sinrhizobium melilti are used in this study. The results showed, these two strains ofA. tumifaciens bacteria were resistant to Trimetheprim and sensitive to chloromphinicol while the S.melilti bacteria gave the opposite of these results,and used as genetic markers for conjugation as one of the methods used in the technique of Horizontal gene transfer, with the use of acardine orange to cure the bacteria from plasmid.Two conjugation procces were performed, between Agrobacterium tumefaciens (AtMcol) and S. melilti, and between Agrobacterium tumefaciens (C58C1)and S.melilti, result showed succecful of conjugation procces with conjugation frequency 1.8x10-2 and 2.1x10-2 respectively, and the transconjugant bacteria result from procces succeded in infecting the root tip of alfalfa seedlings to form root nodules. Characterization of plasmid DNA content for bacterial isolates belonging to conjugated cells, were carried out by using agarose gel electrophorsis and the results showed isolate the presence of discrete clear and isolated band of similar size present in agaros gel.

Keywords: Conjugation technique, Biochemical, DNA

Introduction

Horizontal genes transfer between different organisms, including bacterial communities (Amabile-Cuevas & Chicurel, 1992),has a specialized role in the development of microorganisms, although it is difficult because of genetic control (Lorenz & Wackernagel, 1994),there arethree main mechanisms (Transformation, Transduction and Conjugation) of genetic exchange in bacteria are collectively calledHorizontal genes transfer (Prescotta et al., 2007).The conjugation phenomenon is one of the important technologies for genetic or phenotypic changes that contribute to the development of microorganisms in various environmental complexes such as soil and water by transferring genetic information from Donor cell (F +) to the Recipients cell (F-) (Williams et al. , 1996).The plasmids have the ability to control the process of association with transgenic factors of plasmid DNA called transfer genes, which act to encode the formation of sexual filaments (Pili) on the wall of the donor cell when conjugation process between(F +) and (F-) to connect the two cells forming the so-called Conjugation bridge or the fertilization tube (Pilner, Fullner et al., 1996),to pass through one of the two plasmid DNA strips into the cell (F-),The transmission rate of the chain is about 47Kbp / min (Hartwell et al., 2000), the transfer begins after one of the two DNA strips breaks down with the endonuclease enzymes encoded by the mob gene to generate a DNA sequence (3 ', 5'),then the broken chain travels at the origin of transfer (oriT) from the end of 5 'of the cell received through the conjugation bridge (Willetts &Wilkins, 1984; Strohmaier et al., 1998),Which act as a template for making the complementary tape and bind to the plasmid to return to its normal shape a circular double-chain molecule (Bradley, 1981).This process has broken the barrier of specialization by transferring the trait of a bacterial type to another type, and resulted in the receiving cell genetic units that

encode the desired form as resistance to many antibiotics that are responsible for the accumulation and multiplication of resistance determinants in the genetic material of bacterial cells (Lujan et al., 2007).

Materıals and Methods

Bacterial Cultures

One local isolates of A.tumefaciens (Atmco1) which is isolated by (Al-Zaidy et al., 2013) from crown galls formed on stem of Myrtus communis, A.tumefaciens (C58Cl) obtained from (Ugen-VIB Research Belgium). S.meliloti which is isolated from nodules of Medicago stiva plants.A.tumefaciens (Atmcol) grown on Agrobacterium mannitol medium (AM) (Al-Zaidy et al,. 2013) while A.tumefaciens (C58Cl) was grown on yeast extract bef medium (YEB) (Lichlein & Draper, 1986) and S.meliloti on yeast extract medium (YEM) medium (Vincent, 1970).

Antibiotics Used

Twelve types of antibiotics were used in this study;Ampicillin (AMP):10 □g /ml,Amoxicillin (Amo):25 □g /ml,Cefotaxime (Cef)(Cef):30 □g /ml,Gentamycin (Gen):40 □g /ml,Tetracycline (Tet):30 □g /ml,Trimthprim (Tri):30 □g /ml.

Curing of Plasmids

The standard method was to curing the plasmid DNA content using orange acardine dye (Tomoeda et al., 1974).

Bacterial Conjugation

One volume (1)ml of A.tumefaciens suspension and similar volume of S.meliloti suspension were mixed with a same volume of liquid YEB,AM. These mixtures were incubated in for three houres at (28)℃ , then (0.1) ml of bacterial mixture were spread on the surface of agar solidified YEB,AM this media was supplemented with Chl (35)□g/ml and Tri (30)□g/ml selection of these types of antibiotics depend on the bacterial sensitivity or resistance to those types of antibiotics.This was detected by adding the antibiotic to the agar solidified culture medium, then 0.1 ml of the bacterial suspension was streaked on the surface of the media.

Biochemical Testing of Conjugated Bacteria

The biochemical tests of wild bacteria isolated from crown galls of Myrtus communis were conducted according to Berges Manual of Determinative Bacteriology (Holt et al., 1994; Moore et al., 1988).The tests included; fluoride, acid production of melitzite and production of 3-quito lactose.

Determination of Protein Content

Protein content of S.meliloti and and the transconjugant bacteria was evaluated by Lowry et al. (1951) and modified by Schacterle & Pollack (1973).

Isolation and Purification of Plasmid DNA

Plasmids were isolated by Accuprep ® Plasmid Mini Extraction Kit (Kit-3030, K-3030-1) supplied by the companyBIONEER KOREA.

Determination of Concentrations and Purity of Isolated Plasmid DNA

DNA concentration and purity were calculated by measuring UV absorbance by the spectrophotometer using DNA (Sambrook et al., 1989).

Electrophoresis of Plasmid DNA Models

Plasmid DNA content was described and detected For A. tumefaciens and S. meliloti Wild type, genetically modified and resulting from conjugation with the technology of Agarose gel electrophoresis, according to Al-kanaany et al., 2012).

Results and Discussion

Resistant to Agrobacterium and Rhizobium isolates of Antibiotics

After detection of the sensitivity or resistance of A. tumefaciens (AtMcol) isolated from crown galls of Myrtus communis plants (Zaidi, 2017) and the comparison sample A. tumefaciens (C58C1) and S. meliloti isolated from M.sativa plants of the antibiotics under study, AtMcol showed resistance to most antibiotics (Cip, Chl, Str, Ert, Tri, Tet, Rif, Gen, Am & Amp) and antibiotic sensitivity (Kan, Cef). These results were identical to the A. tumefaciens comparison sample (C58C1) Antibiotics (Cip, Chl, Tet, Cef, Kin), either S. meliloti bacteria also showed resistance to Cip, Chl, Str, Ert, Tet, Rif, Cef, Amo & Amp) and their sensitivity to three antigens (Kan, Rif, Gen, Tri) as shown in table 1.

Table 1. Testing the sensitivity of isolated and transgenic A. tumefaciens and S.meliloti antibiotics

Antibiotic	*S.meliloti*	*A. tumefaciens*	
	Rh1*	(AtMcol)	(C58C1)
Amp	R	R	R
Amo	R	R	R
Cef	R	S	S
Gen	S	R	R
Tet	R	R	S
Tri	S	R	R
Ery	R	R	R
Rif	S	R	R
Str	R	R	R
Chl	R	R	S
Cip	R	R	S
Kan	S	S	S

The Curing of DNA Plasmid Content in A. Tumefaciens Isolated and Transgenic Bacteria

Several concentrates of the acridine orange (250, 500, 750, 1000) µg / mL were used. The final concentration (1000 µg / ml) was the lethal concentration of isolated and transgenic A. tumefaciens,When half of this concentration (500 µg / ml) ofacardine orange is used to curing the Aroobacterium,Showed high efficiency in the process of curing and removal of DNAplasmid content for antibiotic resistance,The highest percentage (82%) for curing was for Rif in isolates A. tumefaciens C58C1, were lowest (18%) for Ery and Amp antibiotics,The isolation of A. tumefaciens (AtMcol) showed the highest curing rate (78%) for the antibiotic Rif,As well as giving the colonies of this isolation the lowest percentage in the loss of resistance status amounted to (21)% towards Ery and the results were different for the rest of antibiotics as shown in the results of Table (2).

Table 2. Curing the resistance of bacterial colonies to antibiotics using acardine orange

Isolates bacteria	**No. Of growing colonieson** Master plate	**No. Of curing colonies (%)** Antibiotics(□g /ml											
		Amp (10)	**Amo (25)**	**Cef (30)**	**Gen (40)**	**Tet (30)**	**Tri (30)**	**Ery (15)**	**Rif (250)100**	**Str (25)**	**Ch1 (35)**	**Cip (30)**	**Kan (30)**
A. tumefaciens (AtMcol)	100	18	19	S	56	20	R	18	78	20	67	50	S
A. tumefaciens (C58C1)	100	23	26	S	74	S	R	21	82	68	S	S	S

The conjugation between S.meliloti (as donor cells) and Wild and transgenicA. tumefaciens (as received cells)

The results in Table (3) indicate the success of the transmission of antibiotic resistance status from the donor to the recipient in terms of genetic characteristics of the conjugant bacteriaTransconjugant Agrobaterium (AtMco1) andTransconjugant Agrobaterium (C58C1)Resistance to (Chl, Tri)As well as reducing the number of colonies after purification from (19) to (13) colony and from (14) to (10) colony and reach the frequency of association (1.8 × 10-2) and (2.1 × 10-2) in S. meliloti bacteria With A. tumefaciens (AtMco1) and S. meliloti bacteria with A.tumefaciens (C58C1) respectively.

Table 3. Bacterial coupling between S. meliloti (as donor cells) and wild and transgenic *A. tumefaciens* (as cells received)

Antibiotic sensitivity test		**The genetic characteristics of the associated A. tumefaciens colonies**	**Number of colonies associated**		**Frequency pairing** $10 \times ^{2-}$
donor cells	**received cells (curing)**		**Before purification**	**After purification**	
S. meliloti Chl^R Tri^S		*A.tumefaciens (AtMcol)* Chl^S Tri^R (Curing)	19	13	1.8
S. meliloti $Chl^R Tri^S$		*A.tumefaciens* (C58C1) Chl^S Tri^R (Curing)	14	10	2.1

Biochemical tests of conjugated bacteria

The conjugated bacteria Transconjugant Agrobacterium (AtMcol) and Trans conjugant Agrobacterium (C58C1) showed the ability to produce 3-ketolactose and to produce the acid from melizitoS and its growth on (YEB, AMM) medium,However, it did not have fluorsens as shown by the results of Table (4),But they succeeded in forming the root noduls,While lacking the ability to form crown galls, on both fertilized Alfalfa seedlings.

Table 4. Biochemical tests of the associated bacteria Tranconjugant bacteria

Bacteria	Biochemical tests				
	Fluorsens test	produce 3-ketolactose	produce the acid from melizitoS	growth on AMM	growth on YEB
S. meliloti)control(	+	–	–	–	–
Agrobacteruim tumefaciens)control(	–	+	+	+	+
Agrobacteruim tumefaciens (curing)	–	+	+	+	+
Transconjugant Agrobacteruim (AtMcol*)*	–	+	+	+	+
Transconjugant Agrobacteruim (C58C1*)*	–	+	+	+	+

Formation of root nodules and protein content in M. sativa seedlings fertilized with Sinorhizobium meliloti and Transconjugant Agrobacterium

The root hairs of the *M. sativa* seedlings were shown with both *S.meliloti* (Comparative +) and Transconjugant Agrobacterium (AtMcol) and Transconjugant Agrobacteruim (C58C1),Different changes are transformed from the straight shape to the distorted shape in succession,The fertilized seedlings responded to the formation of the nodules, which varied in their forms between spherical, oval and branching ovaries, and composition of 40% and 3 node / plant on the side roots in the case of seedlings fertilized with Transconjugant Agrobacterium (AtMcol),While the form of spherical or oval and occurred on the side roots in the case of Transconjugant Agrobacteruim (C58C1) and a composition of 50% and the rate of 2 nodules/ plant,Compared to the positive sample infected with bacteria *S.meliloti*, which amounted to the composition of the contract 72% and the rate of 3 nodules/plant,Which led to the increase in the vegetative content in the total vegetative protein content of theseedlings that formation. The nodules to (4.5, 4.1 and 3.8) respectively Table(5) and figure 1, This indicates the transmission of Sym-plasmid symbiosis from *S.meliloti* to the Acrobacterium in a conjugated manner,. *A. tumefaciens* (AtMcol) and *A. tumefaciens* (C58C1) did not show any ability to form root nodules befor conjugation.

Table 5. The formation of root nodules on the roots of *M. sativa* seedlings by the bacteria associated with Transconjugant Agrobacterium

Bacteria	Number of Plants produced nodules	Rate the number of nodules/plant	Plants produced nodules (%)	The formof the nodules	Location of nodules	protein content)mg/g(
S. meliloti)Comparative +(	Plants produced nodules	3	72	ovaries	The main& lateral	4.5
A. tumefaciens (C58C1))Comparative -(	0.0	0.0	0.0	—	—	—
Transconjugant Agrobacteruim (AtMcol*)*	10	2	40	)spherical, oval and branching(	lateral	4.1
A. tumefaciens (AtMcol))Comparative -(	0.0	0.0	0.0	—	—	—
Transconjugant Agrobacteruim (C58C1*)*	8	3	50	)ovaries(	lateral	3.8

Rate 20 seedlings / treatment

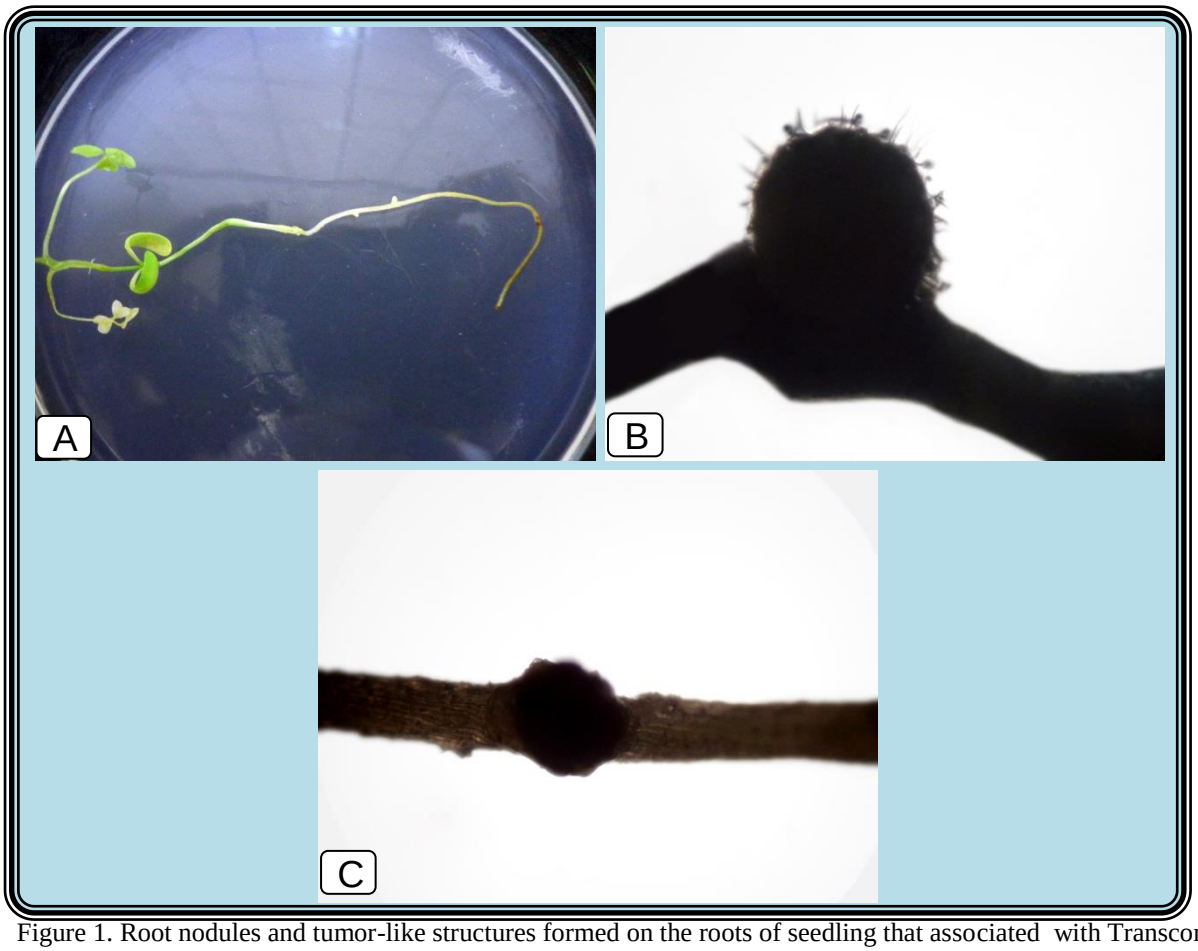

Figure 1. Root nodules and tumor-like structures formed on the roots of seedling that associated with Transconjugant Agrobacterium

A. Root nodules formed on the main root of the transconjugant Agrobacterium (AtMco1).
B. Root nodules of A at force (× 40).
C. Root nodules formed on the lateral root of the Transconjugant Agrobacterium (C58C1) at force (40x).

Determination of the values and purity of plasmid DNA concentrations in S. meliloti and A. tumefaciens bacteria

plasmid DNA concentrations and their purity from associated bacterial isolates obtained from conjugation were measuredand compared with theDNA plasmid concentrations of isolates received,In table (6) (37), there is a marked increase in plasmid DNA concentrations isolated from the received bacteria that have been cured prior to the conjugation process. This indicates the acquisition of nucleic acid from the donor bacteria in the conjugation process, confirming this and supporting the success of the bacterial coupling. The rate of increase in the values of plasma plasmid concentrations in the conjugated bacteria resulting from coupling operations in this study ranged from 44.5 µg / ml to 46.2 µg / ml, The highest and lowest mean of this concentration was by the bacteria resulting from the association between the Acrobacterium conjugated with *S. meliloti* and *A. tumefaciens* (AtMcol), which are associated with *S. meliloti* bacteria respectively,he highest concentration of DNAplasmid prior to curing and conjugation was recorded by *S. meliloti* and reached 120 µg / ml and below in *A. tumefaciens* (AtMcol), which was 61.1 µg / ml, as is evident in countries.

Table 6. Values of DNAplasmid concentrations isolated from *S. meliloti* and *A. tumefaciens* bacteria, which are derived from conjugation

Bacteria	A1	A2	A1/A2	Concentrations) µg / ml(
A. tumefaciens (C58C1)	0.113	0.096	1.173	113
A. tumefaciens(C58C1)المحيدة	0.508	0.344	1.479	50.8
Transconjucant agrobactrium (C58C1)	0.970	0.616	1.575	97
A. tumefaciens (AtMc01)	0.611	0.496	1.232	61.1
A. tumefaciens (AtMc01)المحيدة	0.299	0.204	1.462	29.9
Transconjucant agrobactrium(AtMc01)	0.744	0.511	1.455	74.4
S. meliloti	0.120	0.107	1.119	120

Characterization of DNAPlasmidcontent of S. meliloti and A. tumefaciens of wild and transgenic genes associated with transduction

The plasmid DNA content of these isolates was characterized by the transfer technique on the agarose gel (0.8%),Figure (2, 3) (20.19) showed the separation of plasma plasmid beams in the poles of the electrical transfer of the plasmid content of the two isolates associated with Transconjucant Agrobacterium (AtMcol) and Transconjucant Agrobacteriunm (C58C1) and free the isolation of the two Agrobacterium wild and standerd of plasmid when treated with acardine orange, These resultes appear transferred psym from the donor isolates, to the received isolates, multiplied into the receiving cell, expressed themselves by antibiotic resistance, as well as the pathogenesis of the cell receiving the ability to form Root nodules on fertilized seedlings.

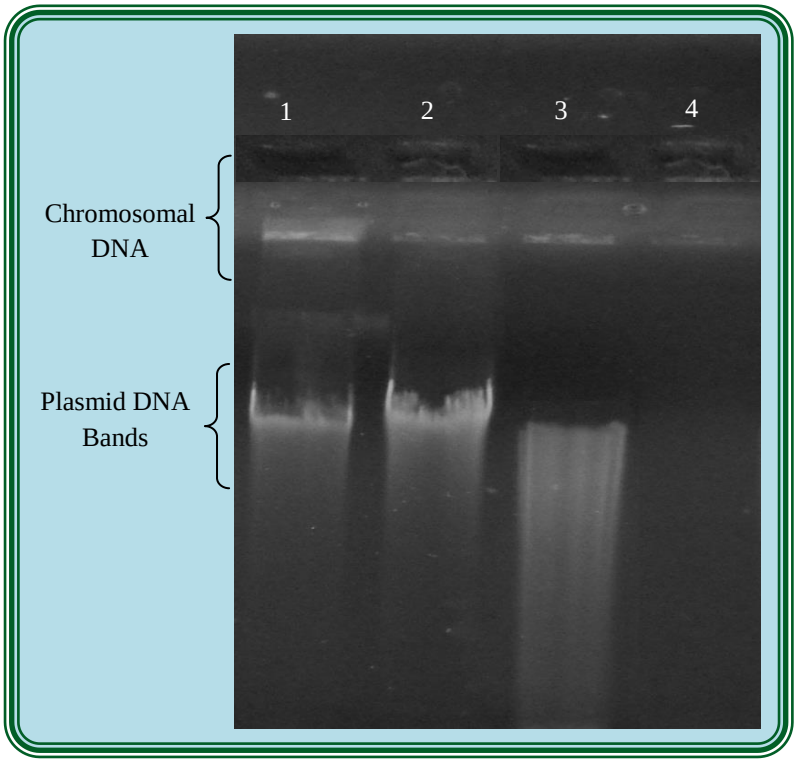

Figure 2. (19) Electroforeses of DNA plasmid content in 0.8% agarose gel of the bacteria resulting from the conjucation between Sinorhizobium meliloti and Agrobacterium tumefaciens (AtMcol)

Column 1. Plasmid content of Sinorhizobium meliloti bacteria
Column 2. Plasmid content of Transconjucant Agrobacterium (AtMcol)
Column 3. Plasmid content of Agrobacterium tumefaciens (AtMcol)
Column 4. Plasmid content of Agrobacterium tumefaciens (AtMcol)

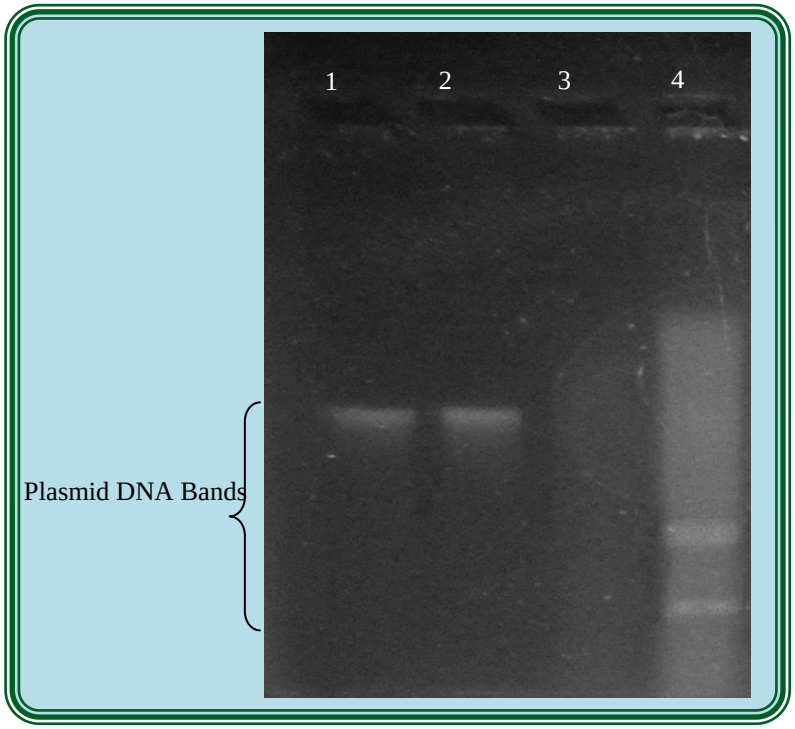

Figure 3. Electroforeses of DNAplasmid content in 0.8% agarose gel of the conjugated Sinorhizobium meliloti and Agrobacterium tumefaciens (C58C1)

Column 1. Plasmid content of Sinorhizobium meliloti bacteria.
Column 2. Plasmid content of Transconjucant Agrobacterium (C58C1).
Column 3. Plasmid content of Agrobacterium tumefaciens (C58C1).
Column 4. Plasmid content of Agrobacterium tumefaciens bacteria (C58C1).

Discussion

(Marja et al., 2004) is considered to be one of the most important bases of application in the diagnostic side, as well as the adoption of biologic aspects and biochemical tests as important evidence in proving the case of horizontal transmission of co-plasmids Sym plasmid by the technique of bacterial coupling between S. meliloti and A. tumefaciens isolated and genetically engineered ("refrences strain"). The results of the resistance of the bacterium Rhizobium antibiotic matched Kücük and Cevheri (2012) in resistance to isolated isolates of alfalfa of Ampicillin, Streptomycin and Chloramphenicol, and adapted to live in the presence of many substances in the soil produced by other microorganisms found with them as fungi and actinomycin Which are characterized by their production of antibiotics, Prompting these bacteria to develop mechanisms for self Mtaat_khassh antibiotic resistance so as to ensure its survival and continuity (Cho et al., 2009). Also, the resistance shown by these bacteria Bjnisseha to ampicillin attributed to the production of the enzyme β-Lactmases analyst for this antibiotic by breaking the loop β-Lactams constituent (Brody et al., 1994), Resistance to risobium for Streptomycin, Amoxicillin, Chloramphenicol, Ampicillin, and Tetracycline was consistent with several studies of this type of antibiotic resistance in a number of isolates of S. meliloti and Rhizobium leguminosarum (Belachew, 2010) Interpretation of its resistance to inhibiting these bacteria inhibits the production of the protein stimulated by these antibodies within the bacterial cell (Talaro & Talaro, 1996).

According to one study, the possession of A. tumefaciens for resistance to Rifampicin, Gentamycin and Tetracycline is often due to the presence of resistance genes in the genetic repertoire of these bacteria (Hellens et al., 2000),While their sensitivity to antagonists Kanamycin, Chloramphenicol and Tetracyclin are explained by their effectiveness in inhibiting the growth of bacteria by preventing them from protein synthesis and cell membrane formation (Shishido & Pepper, 1990),One study has shown that Chloramphenicol, Gentamycin, and Tetracycline inhibit protein synthesis when linked to risobium due to the failure of translation of mRNA during protein synthesis stages in the bacterial cell (Yassin et al., 2005).

Disscution

The resistance shown by the bacteria under study for Ampicillin is attributed to its production of β-Lactmases, which dissolves this antibiotic by breaking its constituent β-Lactams (Brody et al., 1994), The results of resistance to Rhizobium for Streptomycin, Amoxicillin, Chloramphenicol, Ampicillin, and Tetracycline were consistent with several studies in the possession of this species of bacteria as resistant to these antibiotics in a number of isolates belonging to the bacteria S.meliloti and Rhizobium leguminosarum (Belachew, 2010), Whose resistance to inhibiting these bacteria is due to inhibiting the synthesis of the protein stimulated by these antibodies within the bacterial cell (Talaro and Talaro, 1996). One of the studies reported that A.tumefaciens for resistance to Rifampicin, Gentamycin and Tetracycline were due to the presence of antibiotic-resistant genes in the genetic repertoire of these bacteria (Hellens et al., 2000). The sensitivity of Kanamycin, Chloramphenicol and Tetracyclin antibiotics , inhibit the growth of bacteria by inhibiting the process of protein synthesis and cell membrane formation (Yassin, et al., 2005).

The frequency of loss of plasmids can be increased by exposing the cells to compounds that anchor themselves between DNA bases, in particular acridines such as Ethidium Bromide and Acridine orange or by use of less dangerous substances, Sodium dodecyl sulphate (Mickelsen et al., 1985), which results in plasmid-free cells and their growth with antibiotics (Dale and Park, 2004). The results of the high efficiency of acardine orange on curing were consistent with one of the studies, which indicated the efficiency of both the orange acridine and SDS in conjucation the plasmid DNA content of the fertility factor F + in E. coli bacteria, and its effect in reducing the plasmid multiplication and amplification (Hohn & Korn, 1969) and the removal of antibiotic resistance plasmids as well as its effectiveness in removing the F-plasmid factor responsible for bacterial conjugation in Klebsiella spp isolates (Rasool et al., 2003).

The case of the horizental genes transfer of plasmids from the donor cell S.meliloti to Acrobacterium (as received cells) was indicated by Hellens et al. (2000), based on the results of the antibiotic susceptibility test, and the resistance of the isolated and genetically engineered Transconjugant Agrobactrium Chloramphenicol ,this is a clear indication of the possibility of the transfer of the encoded genes to the resistance of this antibody located on the plasmid DNA (R-Plasmid) from the plasmid S.meliloti to A.tumefaciens across the bridge of conjugation between the two cells and multiplied by the process of rolling-circle replication (Talaro and Talaro, 1996).

The initial data obtained in this study recorded the frequency of conjugation of S.meliloti with AtMco1 A.tumefaciens (1.8 × 2-10) and A.tumefaciens (2.1 × 2-10), while studies indicated that the frequency of conjugation to (2 ×10-6) between the different types of Rhizobaceae risobium bacteria, and 10-1 between the different strains of R.meliloti and E. coli PRK2 plasmid bearing the encoded genes of the resistance to tetracycline using KR2 Cosmid for plasmid (1996 Herrer-Cerrera et al., 1998), this variation may be due to the difference in response to wild isolation from genetically engineered conjugation.

Transconjugant Agrobacterium (C58C1), Transconjugant Agrobacterium (AtMco1) succeeded in establishing a symbiotic relationship with the alfalfa plants resulting in the disformation of the root hairs and the formation of a root nodules similar to the contract formed in the specialized relationship between the alfalfa and S.meliloti ,and nitrogen fixed in terms of increased protein content total of vegetative of fertilized plants. This formation of the nodules explains the success of the conjugation process in this aspect in the transfer of pSymA plasmids, the genes responsible for nodules formation (nod genes) and nitrogen fixation (nif genes), and pSym B plasmid , the carrier of cluster genes that encode the construction of exoplysaccharide (Dominguez-Ferreras et al., 2006; Ann et al., 1985). The transformation of the nod genes from S.meliloti to A.tumefaciens and the emergence of these nodules are a clear demonstration of the success of the lateral transformation of these genes by cojugation technique. These results were consistent with one of the studies (Wong et al., 1983) in the success of expression in both S.meliloti and Transconjucant Agrobacterium in the formation of Infection threads and nodules on the fertilized alfalfa seedling.

The results of the plasmid DNA migration on the agaros gel confirmed the success of curing of A. tumefaciens when using acridine orange, in terms of the absence of plasmid DNA bundles on the agaros gel compared with the non-curing isolates, which contain most of the antibiotic resistance genes, as well as the constituent genes of the crown gall of the A.tumefaciens bacteria, which are demonstrative evidence that clearly supported the status of curing prosses. this may be due to the ability of this compound and other nitroacridines, aminoacridines, and ethidium salts to penetrate into the DNA (Hahn, 1976) between the DNA bases of the bacterial cell and interfere with various vital functions such as replication, replication, displacement (Lee et al., 1996), this compound showed high susceptibility to curing plasmids of Pisum sativum, Lens culinans, Sesbania aculeate, Vigna

mungo, Phaseolus vulgaris and loss of resistance to Streptomycin, Amoxicilling and Ampicillin (Naher et al., 2012).

Studies indicate that most genes related to symbiotics relationships that occur on plasmids can be acquired horizontally (Hooykaas et al., 1981), therefore, the transfer of plasmids of the genome of Rhizobium in being studied, when the conjugation process is performed after the treatment of orange acardine cells, possibly due to the movement and transition of pSym from the donor cells to the cells received, ,this was confirmed by the results of the plasmid DNA samples on the agaros gel, which showed a clear variation in the molecular weights of the plasmids in the receiving and donor bacteria,with matching the molecular weights of the plasmids in both the donor and conjugate bacteria, If these plasmids do not have this trait, there may be elements transporter or smaller plasmid (mechaplasmid) that enter into these plasmids are responsible for the movement and transition and to give them new properties of the cells received as antibiotic resistance (Hynes et al., 1986).

References

Amabile-Cuevas,.F. and Chicurel,M.E.(1992) Bacterial plasmids and gene flux.Cell.70:189-199.

Al-Kanaany ,C. F; Hisham, F; Al-Yassri. and Al-Mousawi,A. (2012). Plasmid profile of two isolates of Sinorhizobium meliloti isolated from different soil areas in Basrah Iraq. Bas. J. Agr. Sci., 25: 13-18.

Ann,M.Hirsch; Daniela drake.; Thomas W.Jacobs; and Sharon R.Long (1985). Nodules are induced on alfalfa Roots by Agrobacterium tumefacienes and Rhizobium trifolii containin small segments of the Rhizobium meliloti nodulation region.Vo: 161 No(1):223-230.

Bono,J.J.; Gressent,F.; Niebel, A.; Cllimore, J.V.; and Rnajeva,R.(1996). Biochemical characterization of nod factor binding sites in Medicago roots cell suspension cultures. In: Biology of Plant Microbe Interaction. (eds.) Stacey, G.; Mullin, B.and Gresshoff, P.M.) International Society for, Molecular Plants Microbe Interactions,St.Paul,M.N.,P. 90-104.

Belachew,T. (2010). Intrinsic antibiotic resistance, survival of Rhizobium leguminosarum strains and fixation potential of pea varieties (Pisum sativum L.) In southeast Ethiopia. Int. J. Mic. Res., 1: 75-79.

Brody,T.M; Larner,J.; Minneman,L.P. and Nen, H.C.(1994). Human Pharmacology. 2nd ed., Mosby- year book,Inc., St. Louis, Missouri.

Bradley,D.E.(1981)conjugative pili plasmids in Eschericah coli K-12 and Pseudomonag species,P.217-226. In S.B Levy,R.C.Clones and E.L.Koenig (ed).Molecular biology,pathogenicity gand ecology of Bacteria plasmids.plenum press, NewYork.

Cho,H.;Pinto, U. M. and Winanse,S.C.(2009). Transsexuality in the rhizosphere: quorum sensing reversibly converts Agrobacterium tumefaciens from phenotypically female to male.J.bacteriol.,191, 3375-3383.

Dominguez- Ferreras, A.;Perez-Arnedo, R.;Becher ,A.;Olivares ,J.;Soto, M .J.and Sanjuan ,J.(2006). Transcriptome profiling revents the importance of plasmid sym B for osmoadaptation of Sinorhizobium meliloti,J.Bact.,188: 7617-7625.

Dale,J.M. and Park, S.F.(2004). Molecular Genetics of Bacteria. 4th ed,John Wiley and Sons,Ltd. Chichster, England.

Fullner, K.J.; Cano,L.J.and Nester,E.W. (1996). Pilus assembly by Agrobacterium T- DNA transfer gene. Science, 273: 1107- 1109.

Guo,X.;Flores,M;Mavinguip,F.; Hernandez, G;Davila, G.and Palacios, R. (2003). Natural genomic design in Sinorhizobiummeliloti novel genomic architecture.Genome Res .,13:1810-1817.

Hartwell,L.; Hood,L.; Glodberg,M.L; Reynold,A.E; Silver,L.M and Vers,R. C.(2000). Genetics from genes to genemes. The McGraw – Hill companies, Inc,Boston,U.S.A.

Holt, J.G.; Krieg ,N.R.; Sneath ,P.H. ;Slaley, J.T.and William, S.T. (1994). Bergey's manual of determinative bacteriology 9th edu. Williams and Wilkins Baltimors, Netherlands. html, cited 22 Jan 2009.

Hellens,R.; PhilifMulliaeux. and Harry, K.(2000). A guide to Agrobacterium binary Ti Vectors trends in plant. Science, 5(10): 446-451.

Hohn,B.and Korn,D.(1969).Co-segregation of sex factor with the Escherichia coli. Chromosome during curing by acridine orange. J. of Molecular biology, 45:385-395.

Hahn,F.E. (1976). Elimination of plasmids from bacteria. Antibiotics and Chemotherapy,20, 196-201.

Hellens,R.; PhilifMulliaeux. and Harry, K.(2000). A guide to Agrobacterium binary Ti Vectors trends in plant. Science, 5(10): 446-451.

Herrera-Cervera,J.A.; Olivares,J.; and Sanjuan,J. (1996). Ammonia inhibition of plamid PRme GR4a Conjugal transfer between Rhiobium meliloti stains. Appl.Environ.Microbiol.,62:1145-1150.

Hooykaas,P.J.; Van Brussel,A.A.; Dulk-Ras,H.; Van- Slogteren,G.M. and Schilperroot, R.A. (1981). Sym plasmid of Rhizobium trifolii expression in different rhizobial species and Agrobacterium tumefaciences. Nature, 91:351-353.

Hynes, M. F.; Simon, R.; Muller, P.; Niehaus, K.; Labes, M and Puhler, A. (1986) .The two megaplasmids of Sinorhizobium meliloti are involved in the effective nodlation of alfalfa. Mol .gen. Genet., 202; 356-362.

Hiroki, N.; Toshiki, C.;Kenichi ,K.;Akihiro, S; Shiro, H and Nikiko, A.(2008). Transposon mediation allows symbiotic plasmid of Rhizobium leguminosarum bv.trifolii to become a symbiosis island Agrobacterium and Rhizobium.. The Journal of General and Applied Microbiology, 54: 107-118.

Hirsch,A.M .(1992). Development biology of legume nodulation . New Phytol., 122: 211-237.

Hirsch,A.M.H.; Daniela,A; Thomas, W;Jacobs and Sharon, R.L. (1985). Nodules are induced on alfalfa roots by Agrobacterium tumefacienes and Rhizobium trifolii containin small segments of the Rhizobium meliloti nodulation region.J. Bacteriol., 161:223-230.

Kondorosi, A.; Kondorgi, E.; Pankhurst, C. E.; Broughton,W. J. and Bauflav, Z.(1982). Mobilization of Rhizobium meliloti megaplamsmid curring nodulation and nitrogen fixation genes into other Rhizobium and Agrobacterium. Mol. Gen.,188:433-439.

Lee,L.; Huang,Y. and Chen, C.W.(1996). Two classes of ethidium bromide resistant mutants of streptomycess lividans 66. Microbiology, 142: 1041-1047.

Lowry,O.H.;Roisebrough,N.J.;Farr,A.L.and Radad,R.J.(1951). Protein measurment with folin phenol reagent. J. Bio.Chem.,193: 257-265.

Lichtenstein,C.and Draper,J.(1986). "In DNA cloning: Apractical Approach",(ed.)Glover,D.M. (IRL,Oxford,Vol.2).

Lujan, S. A.; Guagas, M.; Ragonese, H; Matson, S.Wand Redingbo, M. R. (2007). Disrupting antibiotic resistance propagation by inhibiting the conjugative DNA relaxase. P.N.A.S., 104:1228-12287.

Lorernz,M.G.and Wackernagel,W .(1994). Bacterial gene transfer. Environment Microbiol.Rev., 58:563-602.

Moore, L.W.; Kado, C.I. and Bouzar, H.(1988). Agrobacterium. In Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. by Schaad, N.W. American Phytopathological Society Press, St. Paul, Minn. pp. 16–36.

Marja , K. C., William, R., Horwath, B. D.and Hammock,W.(2004). \Isolation of a strain of Agrobacterium tumefaciens (Rhizobium radiobacter) utilizing methylene urea (ureaformaldehyde) as nitrogene source .Can. J. Microbiol., 50: 167-174.

Mickelsen, P. A.; Plorde, J. J; Gordon, K. P.; Hargisis,C.and Maclure, J. (1985). Intability of antibiotics resistance in an strain.of Staphylococcus epidermidis isolated from on outbreak of prosthetic value endocarditis.J.Infect.Dis.,.152:50-58.

Mysore, K.S.;Nam, J and Gelvin, S.B .(2000).An Arbidopsis histone H2 Amutantis deficient in Agrobacterium T-DNA- integration.Proc.Natl.Acad. Sci., 97:748-953.

Naher, M.; Zinat, M.; Hasan, M.Z.; Khadiza, Z.and Fahmida , J. (2012). Effects of plasmid curing on Rhziboium spp. J. of Mic., 2: 2074-5346.

Prescotta,L.M.; Harley,J.P. and Kelin,D.A (2007). Microbiology Prokaryotes: Bacterial Genetic System Taxonomy Microbiology. bytes.22, No .5.

Rasool, S. A.; Ahmad,A.; Khan,S. and Wahab,A. (2003). Plasmid bone antibiotic resistance factors among indigenous Klebsiella pneumonia .J.Bot.,35(2) 243-248.

Strohmaier,H.;Noiges,R.;Kotschan,S.;Sawers,G.; Hogenauerg G.; Zechner, E. L.; Koraimann, G.; Zechner, E. L.; Koraimann, G. (1998). Single transduction and bacterial conjugation characterziation of the role of Arc A in regulating conjugation transfer of the resistance plasmid Ri. J.Mol.Biol.,277:309-316.

Schacterle,G.R.,and Pollack,R.L.,(1973). A simplified method for the quantitative assay of small amounts of protein in biological material.Anal.Biochem.sl:654-655.

Sambrook,J.;Fritsch,T.F. and Maniatis,T. (1989). Molecular cloning a laboratory.New York,USA.

Shishido, M. and Pepper,I.L. (1990). Identification of dominant indigenous Rhizobium melilolti by plasmid profiles and intrinsic antibiotic resistance. Soil Bio. Bioch., 22: 11-16.

Talaro, K. and Talaro, A.(1996). Foundation in microbiology. 2nd ed., W.M.C. Brown Publishers,PP :281-282 .

Tomoeda, M.; Inuzuka, M.; Anto, S. and Konishi, M. (1974). Curing action of sodium dodecyle sulfate on a Proteus mirabilis R+ strain. J. Bacteriol., 120: 1158-1163.

Valerie, O. and Sharon, R. L. (1999) .Bacterial genes induced the nodule during the Rhizobium legume symbiosis. Molecular Microbiology, 32(4):837-849.

Vincent,J.M.(1970). A manual for the Practical Study of Root Nodule Bactria.IBP Handbook No.15. Oxford: Blackwell Scientific Publication,Oxford,PP.113-131.

Williams,H.G.; Day, M. J.; Fry, J. C .and Stewart, G. J.(1996). Natural transformation in River epilithon. Appl.Environ. Microiobl., 62:2994-2998.

Willetts, N. and Wilkins, B. (1984) .Processing of plasmid DNA during bacterial conjugation. Microbiologicla Reviews, 48(1):24-41.

Wessells,N.K. and Hopson,J.(1988). Biology Rnadom House,INC.,New York.PP.303-320.

Wong,C.H.; Pankhurst, C. E.; Kondorosi,A. and N. D Broughton, W. J. (1983). Morphology of root nodules and Nodule- like structures formodby Rhizobium and Agrobacterium strains containing Rhizobium meliloti megaplasmid . J of Cell Biology, 97 :787-794.

Xi- Wan; Jan, H.; Allessandra ,L.; Chiara ,G.; Diederikvande ,P.; Elena, F.; Ton, B. and Henk,F. (2007) Medicago trancatula EWoD40-1 and ENOD 40-2 are both involved in nodule initiation and bacteroid development .J. of Experimental Botany., 72: 1-9.

معارج، محمد عبد المحسن.(9991). مقدمة في الهندسة الوراثية، كلية الطب البشري، جامعة الموصل.

الزبيدي، اريان محمد حامد اسماعيل (5002). نقل بلازميدات بكتريا itolilem.S الى بكتريا iloc.E بطريقة تي لة ماجستير، كلية التربية، الاقترانالبكتري والتحول الوراثي ودورها في تكوين العقد الجذرية. رسا جامعة الموصل,العراق.

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