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Transfer of *Rhizobium leguminosarum nod*D1 and *nod*G, and *Azotobacter chroococcum nif*H1 and *nif*V, into *Bacillus megaterium* using physicochemical and molecular techniques.

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ABSTRACT

This study involved two techniques for transferring important genes related to nitrogen fixation into *Bacillus megaterium*. Initially, morphological characteristics and molecular properties were analyzed to identify isolated bacteria. This process led to the isolation of *Bacillus megaterium*, *Azotobacter chroococcum*, and *Rhizobium leguminosarum* using a specific growth medium. Morphologically, the results showed that all the bacterial colonies were circular, convex, and smooth. *A. chroococcum* was creamy color, while *B. megaterium* and *R. leguminosarum* appeared white. The application of molecular approaches relied on identifying chromosomal DNA, specifically the *nif*H1 and *nif*V genes of *A. chroococcum*, and the plasmid DNA, including the *nod*D1 and *nod*G genes of *R. leguminosarum*. Additionally, genomic DNA and two primers of *B. megaterium* were included. The *nod*D1 and *nod*G genes were successfully transferred from *R. leguminosarum* to *B. megaterium*, resulting in 46 colonies on agar plates of Sperber medium that contained tetracycline and ampicillin as genetic markers. Furthermore, the *nif*H1 and *nif*V genes were transferred into the transformant *B. megaterium* from *A. chroococcum* via a conjugation mechanism, leading to the growth of 170 colonies on plates of Sperber's agar medium containing erythromycin and ampicillin as genetic markers. To confirm the success of these transfers, molecular analyses were conducted to detect the *nod*D1 and *nod*G genes in the transformant *B. megaterium*, as well as the *nif*H1 and *nif*V genes in the conjugant *B. megaterium*. This was achieved using appropriate primers and PCR conditions for all the genes, followed by gel electrophoresis, demonstrating the successful completion of both techniques..

***Keywords*:** Nitrogen fixation genes, Bacterial transformation, Bacterial conjugation, PCR, Gel electrophoresis.

INTRODUCTION

In the rhizospheres of plants, *Azotobacter* and *Rhizobium* interact with a diverse range of microbes, and the presence of these other microbes can occasionally stimulate these interactions, enhancing the growth response of the host plant [1, 2]. Both *Rhizobium* and *Azotobacter* are recognized as effective nitrogen fixers, functioning both symbiotically and non-symbiotically in succession [3].The biological nitrogen fixation process involves various genes and their products, such as *nif*, *nod*, and *fix*, as well as those responsible for polysaccharide synthesis, host specificity, the infection process, and competition, and this process occurs in strains of *Rhizobium* in partnership with legumes and *Azotobacter*, particularly when nitrogen levels are insufficient [4]. Research has shown that the growth of nitrogen-fixing nodules on legume roots is dependent on the *nod* genes of *Rhizobium* [5]. All *rhizobia* possess the *nod*D gene [4], which is considered a primary signalling protein that responds to plant flavonoids. It binds to *nod* boxes, which are the upstream binding sites of *nod* genes such as *nod*B and/or *nod*A, this binding triggers a cascade of *nod* gene expression, leading to the formation of *nod* factors [6]. The *nod*D1 gene, which appears before the *nod*ABC operon, plays a crucial role in the nodulation process [7]. Additionally, the *nod*G gene is important for establishing nodulation [8]. Both symbiotic and free-living diazotrophs can fix nitrogen and rely on *nif* genes for transcription [9]. Low levels of fixed nitrogen and oxygen stimulate the expression of these *nif* genes, and the root environment of host plants actively maintains these low oxygen levels [10]. The development of the iron (Fe) protein complex is influenced by the structure of the *nif*H gene, which is essential for nitrogen fixation [11]. *Nif*H represents dinitrogenase reductase, a necessary component for the maturation of apodinitrogenase and the biosynthesis of FeMo-Co, additionally; *nif*H serves as an obligatory electron donor to dinitrogenase within the dinitrogenase cycle, and *nif*V gene is involved in synthesizing homocitrate, which is also crucial for FeMo-Co synthesis [12]. *Bacillus megaterium* is recognized as one of the biofertilizers that relies on selected strains of beneficial eubacteria and it is used as an effective soil inoculum with the

capability to solubilize phosphate, making it accessible for plants [13]. This bacterium plays a vital role in supporting plant growth by providing readily available forms of phosphorus by producing organic acids and carbon dioxide (CO2), which increase soil acidity and convert insoluble phosphorus into soluble forms [14]. To control bacterial activity and their functions in various fields, researchers have focused more on bacterial genetics and employed several techniques to enhance the ability of bacteria to perform multiple beneficial functions, such as transferring specific genes. Horizontal gene transfer, also known as lateral gene transfer, is a mechanism by which one organism transfers genetic material to another organism that is not its offspring, and this process can lead to significant changes in a bacterial genome and involves various mechanisms, including transformation, conjugation, and transduction [15]. Transformation occurs when a cell receives and expresses new genetic material (DNA), following transformation, the introduction of these new genes typically results in the organism exhibiting a new trait that is detectable, thus, gene transformation essentially means "change," induced by genes, and involves the introduction of one or more genes into an organism to alter its characteristics [16]. A crucial step in the molecular cloning process is the transformation of plasmids into bacterially competent cells [17]. Most circular DNA fragments capable of replication are called plasmids, which are shorter than the bacterial genome. They can transfer genetic information to different species or strains of bacteria, and plasmids often carry multiple resistance genes, which can be found on DNA segments that can move between plasmids or are associated with transposable elements, also known as jumping genes [18]. Conjugation of bacteria is an important process of horizontal gene transfer, in which DNA is transferred from a donor bacterium to a recipient bacterium through direct contact. This mechanism is generally conserved among bacteria. It can be found in a variety of environments, such as surface waters, plants, soil, biofilms, sewage, and in bacterial populations associated with hosts [19, 20]. Conjugation as a mechanism in the laboratory has been demonstrated in Mycoplasma agalactiae, this process involves mixing two strains in a liquid culture marked with antibiotic-resistant genes, leading to a bilateral transformation of DNA, initially, integrative conjugative element (ICE) positive cells transfer a mycoplasma integrative conjugative element (MICE) horizontally to ICE negative cells, additionally, there is a process known as mycoplasma chromosomal transfer (MCT), which involves the movement of large blocks of chromosomal DNA from ICE-negative cells to ICE-positive cells [21,22,23].

This study aimed to utilise physicochemical and molecular techniques to create a new transformant-conjugant strain of *Bacillus megaterium* that carries *nod* and *nif* genes. This genetically modified bacterium can be used as an effective biofertilizer to improve the availability of phosphorus and nitrogen for plants.

MATERIALS AND METHODS:

Study Site:

The current investigation was conducted at the University of Sulaimani's College of Agricultural Sciences in the city of Sulaimani, Iraq. Every bacterium was isolated from Bakrajow's organic farm, and molecular analyses were performed in the laboratories.

Bacterial Isolation:

Healthy, undamaged, firm, and pink nodules from broad beans were utilized to isolate *Rhizobium leguminosarum sp*. using Yeast Mannitol Agar (YMA) medium, as described in reference [24]. Additionally, *Azotobacter chroococcum sp*. was isolated from the same soil using a modified Ashby's medium, in accordance with [25]. Sperber's medium was employed to isolate *Bacillus megaterium* from the exact location, as indicated in reference [26].

Bacterial Purification and Colony Characteristics:

On selective media, actively growing loops from colonies of different bacterial species were streaked. The growth of *A. chroococcum* was assessed after 7 days, while *B. megaterium* and *R. leguminosarum* were checked after 24 to 48 hours of incubation at 28ºC. Once well-separated, uncontaminated colonies appeared on the plates, picking and plating were repeated at least four to five times [27]. The characteristics of the bacterial colonies, including their shape, type, and color, were analyzed based on sources [28, 29].

Bacterial Storage and Maintenance:

Bacterial cultures were stored briefly at 4°C in slant medium, but for a longer duration at -70°C with 20% glycerol [27].

Molecular Identification of Bacteria:

Molecular studies were conducted following the methods outlined in [30] to identify specific bacteria using designated kits, primers, and standard techniques. The Genentech Bio PrimePrep Plasmid DNA kit was employed to extract plasmid DNA from *R. leguminosarum* to identify the *nod*D1 and *nod*G genes. Additionally, the Presto™ Mini gDNA kit was used to extract chromosomal DNA from *A. chroococcum* to detect the presence of the *nif*H1 gene and the *nif*V gene. Furthermore, genomic DNA from *B. megaterium* was analyzed to confirm its presence in the area using primers (1) and (2).

PCR Amplification Conditions :

The PCR conditions for the two primers of *B. megaterium* were conducted according to [31]. The protocols for the *nod*D1, *nif*H1, and *nif*V genes were based on [32], [33], and [34], respectively. For the *nod*G gene, the method was modified from [8] and included a single cycle at 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds, 62°C for 1 minute, and 72°C for 1

minute. The process concluded with a final extension cycle at 72°C for 6 minutes. All primer sequences and their corresponding references are listed in Table 1.

Table1. The primers used in the experiment

Primers	Sequences (5'-3')	Nucleotides	References
*nod*D1- F-	AGAGTTTGATCCTGGCTCAG	20	(32)
*nod*D1- R-	AAGGAGGTGATCCAGCC	17	
*nod*G- F-	GAGCTGACCCATCCGATGA	19	(8)
*nod*G- R-	CGACGACCGAGGTGATGTT	19	
*nif*H1- F-	TTCCATCAGCAGCTCTTCGA	20	(33)
*nif*H1 -R-	GGCAAAGGTGGTATCGGTAA	20	
*nif*V- F-	GATGGCTAGGGTGATCATCGACGA	24	(34)
*nif*V -R-	GCCATTCCTCCTGCCGCCAGTTCG	24	
Random Primer 1	GGT GCG GGAA	10	(31)
Random Primer 2	GTA GTC ATAT	10	

Electrophoresis:

To identify plasmid, chromosomal, and genomic DNA alongside the PCR products, we prepared an agarose gel by dissolving 1.5 g of agarose in 100 ml of TBE buffer using a microwave for 3 minutes. Once dissolved, we allowed the mixture to cool to 45°C at room temperature. We then added 5 µl of ethidium bromide (5 mg/ml) to stain the gel. The gel was placed in a tank filled with TBE buffer to ensure it was fully soaked. Electrophoresis was conducted at a voltage of 80 to 100 V for 2 to 3 hours, depending on the specific product being analyzed, and the gel tank was covered with a lid during this process. Finally, DNA fragments were visualized using a UV transilluminator set to 312 nm. [35].

Antibiotics Resistance Test:

The sensitivity of bacteria to antibiotics was tested using gentamicin, tetracycline, chloramphenicol, ampicillin, cefotaxime, erythromycin, streptomycin, and rifampicin. Each antibiotic was prepared at a stock concentration of 10 mg/ml to achieve the recommended working concentrations of 10 µg/ml for gentamicin and tetracycline, 25 µg/ml for chloramphenicol, 20 µg/ml for ampicillin, 30 µg/ml for cefotaxime, 15 µg/ml for erythromycin, 10 µg/ml for streptomycin, and 5 µg/ml for rifampicin, based on sources [36; 37; 38].

Obtaining Competent Cells of *B. megaterium*:

Bacillus megaterium was prepared as competent cells for the transformation process using a modified procedure [39]. A colony from a new *B. megaterium* plate was suspended in 100 ml of Sperber's broth to create an overnight culture. This culture was maintained at 28 °C for 24 hours while shaking at 100 rpm in an incubator Next, 10 ml of the overnight culture was mixed with 90 ml of fresh Sperber's broth and incubated in a shaking incubator for 90 minutes at 28 °C, until an optical density (OD) of approximately 0.3–0.6 at 600 nm was reached. The cells were then pelleted by centrifugation of 10 ml aliquots at 4 °C for about 10 minutes at 4000 x g. Following centrifugation, the cells underwent a third wash with 5 ml of 0.1 M CaCl2. After washing, the cells were resuspended in 10 ml of CaCl2 and incubated on ice for an hour, after which the supernatant was discarded. The cells were then centrifuged twice. Finally, the cells were resuspended in 2 ml of ice-cold 0.1 M CaCl2 containing 20% glycerol. The competent cells can be stored at -20 °C or transformed immediately.

Transformation Technique:

The transformation procedure was conducted according to the method described in [40]. In this process, 2 µl of extracted plasmid from *R. leguminosarum* was added to 200 µl of *B. megaterium*, which had been prepared as competent cells in an Eppendorf tube. After the colonies appeared and were counted, PCR and gel electrophoresis techniques were utilized to determine whether the nodD1 and nodG genes were successfully transferred to the transformed B. megaterium cells.

Conjugation Procedure:

Based on [41], the processing of the conjugation involved using *A. chroococcum* as the donor cells and *B. megaterium* transformants as the recipient cells. After the colonies developed on the plates, they were enumerated. The transmitted *nif*H1 and *nif*V genes were then examined in the *B. megaterium* transformant-conjugants using PCR and gel electrophoresis techniques.

RESULTS AND DISCUSSION:

This research aimed to develop a new recombinant *Bacillus megaterium* using physico-chemical and molecular transformation methods. This strain incorporates *nod* and *nif* genes to be utilized as biofertilizers, facilitating plant access to phosphate and nitrogen.

Identification of bacteria:

To identify and confirm the presence of bacteria in the location, colony characteristics and molecular analyses serve as screening indicators.

1. Bacterial Isolation and Colonies Characteristics:

At an incubation temperature of 28°C, colonies of isolated *R. leguminosarum* and *B. megaterium* appeared within 24 to 48 hours, while colonies of *A. chroococcum* were observed after 3 to 7 days on their selective media. The colony morphology of *R. leguminosarum* was smooth, convex, white, and circular, consistent with reports from other researchers [42, 24]. The colonies of *A. chroococcum* exhibited smooth, convex, creamy, and circular characteristics. Additionally, distinct *Azotobacter* strains from various locations in the Kurdistan region of Iraq were identified by other studies, all reaching similar conclusions [43, 44, 24]. The *B. megaterium* colonies were also smooth, convex, white, and round, which aligns with findings from other researchers, including [45; 31; 24].

2. Isolation of DNA from Bacteria:

Using molecular approaches and agarose gel electrophoresis, it was clear to identify the plasmid DNA of *R. leguminosarum*, the chromosomal DNA of *A. chroococcum*, and the genomic DNA of *B. megaterium*. The results are shown in Figure 1, specifically in Lanes 2, 3, and 4, respectively.

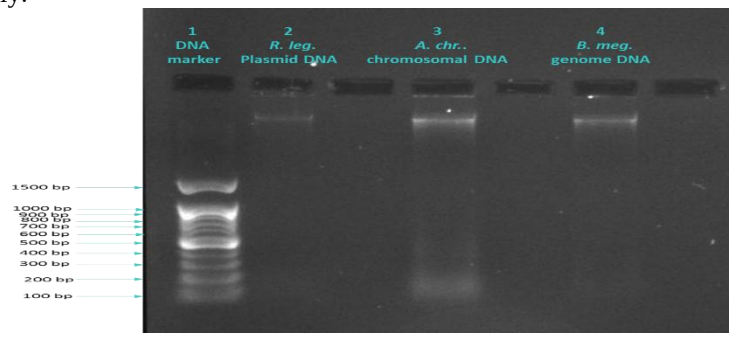

Figure 1. Displayed DNA marker lane (1), plasmid DNA from *R. leguminosarum* lane (2), chromosomal DNA from *A. chroococcum* lane (3), genome DNA from *B. megaterium* lane (4) on agarose gel electrophoresis.

3. Molecular Identification of *Bacillus megaterium*:

Genomic DNA was extracted from *B. megaterium* to confirm its presence at the molecular level using two primers. The results of the PCR showed strong, distinct, and repeatable banding patterns on gel electrophoresis, as illustrated in Figure 2, specifically in Lanes 2 and 3. Using these primers, *B. megaterium* was verified to be present in various locations, as referenced in studies [26, 31, 24].

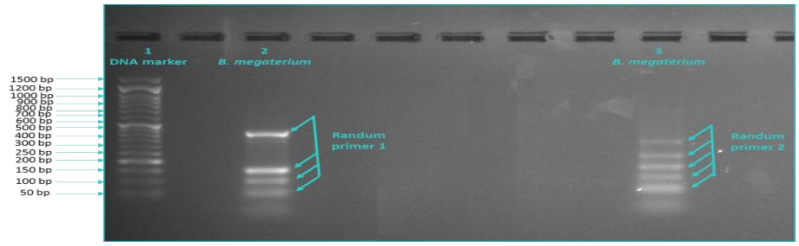

Figure 2. PCR amplification products of *B. megaterium* using two random primers were visualized by gel electrophoresis. (Lane 1): DNA marker; (Lane 2): random primer 1 (+); (Lane 3): random primer 2 (+).

4. Molecular Identification of *Rhizobium leguminosarum*:

The molecular identification of *R. leguminosarum* relied on plasmid DNA extraction to detect the *nod*D1 and *nod*G genes, which appeared as bands of 150 bp and 64 bp, respectively, as shown in Figures 3 and 4, Lane 2. The presence of these *nod* genes on the plasmid is significant [46]. Among the most interesting regulatory nodulation genes is *nod*D, which is constitutively expressed, and in the presence of appropriate plant inducers, typically flavonoids, *nod*D triggers the transcription of additional nodulation genes, initiating the nodulation process. *Nod*D belongs to the LysR family of transcriptional regulators [47; 48; 49], and *nod*D1 precedes the *nod*ABC operon [7]. Many species of *Rhizobium* contain multiple homologues of *nod*D [50, 51], performing divergent roles and expanding the symbiotic host range [52]. *Nod*G is considered one of the host-specificity nodulation genes [53] and is homologous to FabG, an enzyme typically involved in fatty acid elongation, functioning as a 3-oxoacyl-acyl carrier protein reductase [54]. Therefore, the significance of *nod*G and *nod*D1 in nitrogen fixation draws attention to their selection for our study.

The existence of *nod*D in *R. leguminosarum* has been confirmed by several studies [55, 56, 57]. Additionally, a study [24] detected two types of *nod* genes (D2 and D3) on the *R. leguminosarum* plasmid DNA within the same region examined in this research. Furthermore, [58; 59; 60; 61] clarified that the *nod*G gene identified in three *rhizobial* strains (*Rhizobium sp.* N33, *S. meliloti*, *and Mesorhizobium sp*. 7653R) is located within the same operon and is directly adjacent to *nod*FE. Likewise, [8] detected *nod*G in the *Rhizobium tropici* strain PRF 81, matching the size identified here. Finally, [62] found that all *nod* genes were present in all the tested Rhizobium species.

5. Molecular Identification of *Azobacter chroococcum*:

The molecular diagnosis of *A. chroococcum* was conducted by extracting chromosomal DNA to detect the *nif*H1 gene, approximately 700 bp, and the nifV gene, approximately 1146 bp, as shown in Lane 2 of Figures 5 and 6, respectively. This approach is based on the knowledge that the chromosome contains *nif* genes found in certain free-living bacteria [63, 46]. The presence of the *nif*H gene indicates the likelihood of a nitrogenase system being present and suggests the organism's capacity to fix nitrogen; this is because the product of the *nif*H gene is a crucial component of the nitrogenase system and contributes to the formation of the Fe-protein complex [64]. The chromosomal region that encompasses all *nif* genes is located between the fragments that include *nif*H1, H2, and H3, and those that contain *nif*U, *nif*V, and *nif*S [65]. Consequently, the importance of the *nif*H1 and *nif*V genes in nitrogen fixation highlights the relevance of selecting these genes for our research. The existence of the *nif*H gene and the determined sequence align with findings from references [66, 33]. Additionally, the sequence of the nifV gene was confirmed in previous studies on *A. vinelandii* [34] and *Azotobacter chroococcum* [44, 67]. Furthermore, reference [24] identified two types of chromosomal *nif* genes (H2 and H3) in the same region of *A. chroococcum* chromosomal DNA during this investigation.

Gene Transfer Techniques:

The genes studied were transferred using three methods: chemical, physical, and molecular techniques through transformation and conjugation.

1. Transformation Technique:

1.1. Bacterial Antibiotic Resistance for Transformation:

The evaluation results of antibiotic resistance screening indicated that *R. leguminosarum* was resistant to all antibiotics except tetracycline. In contrast, *B. megaterium* was resistant to tetracycline and erythromycin, but sensitive to the other antibiotics. Based on these findings, ampicillin and tetracycline were selected as antibiotic markers for the transformation technique.

1.2. PCR-mediated transfer of *nod* genes between *R. leguminosarum* and *B. megaterium*

Calcium chloride treatment, a simple and equipment-light chemical transformation method [16], is essential for making bacterial hosts competent for genetic transformation since they are not readily transgenic [68]. Bacterial plasmid transformation, a crucial laboratory technique for introducing plasmid DNA carrying foreign genes into cells [69], involves plasmid construction, host cell preparation, and DNA delivery [70]. Transfer the *nod* genes (*nod*D1 and *nod*G) from *R. leguminosarum* into *B. megaterium*, a transformation technique was employed. Following this process, 46 resistant colonies were obtained on Sperber medium supplemented with ampicillin and tetracycline as genetic markers. These findings demonstrated that the transformation between *B. megaterium* and *R. leguminosarum* was successful, as the colonies derived from this technique. To verify the transfer of the *nod* genes, a molecular investigation was conducted to identify *nod*D1 and *nod*G in the *transformant B. megaterium* and to compare them to the *nod* genes in *R. leguminosarum*. Figures (3) and (4) show positive results for *nod*D1 (150 bp) and *nod*G (64 bp) in *R. leguminosarum* (Lane 2) and transformant *B. megaterium* (Lane 4), respectively, while *B. megaterium* (negative control) exhibited negative results in both figures (Lane 3). According to these findings, the plasmids containing *nod* genes, including *nod*D1 and *nod*G, were successfully transferred to the competent cells. Using CaCl2, recombinant plasmids and R-factors were efficiently transferred to *E. coli* cells [71]. This technique has been widely utilized due to its ease of application [17]. Additionally, *nod*C, *nod*D2 and *nod*D3 were also transferred by [72, 73] between the same bacteria using this technique. Furthermore, the transformation process involving a plasmid from *Pseudomonas aeruginosa* and competent cells of *E. coli* was carried out [74]. Moreover, the transformation was achieved by cloning the *SacB* gene from *B. licheniformis* MJ8 into the plasmid pTG19-T, which was then transferred to *E. coli* DH5α [75].

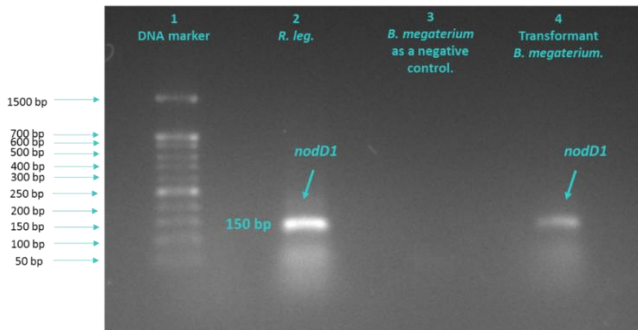

Figure 3. PCR results of *nod*D1 gene with (150 bp): DNA marker (lane 1), *R. leguminosarum* and transformant *B. megaterium* are lanes 2 and 4 respectively (+ve resuls) and lane 3 is *B. megaterium* (-ve result).

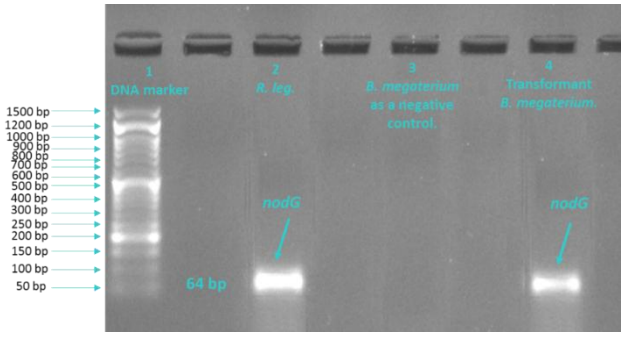

Figure 4. PCR results of *nod*G gene with (64 bp): DNA marker (lane 1), *R. leguminosarum* and transformant *B. megaterium* are lanes 2 and 4 respectively (+ve resuls) and lane 3 is *B. megaterium* (-ve result).

2. Conjugation Technique:

2.1. Bacterial Antibiotics Resistance for Conjugation:

The screening for antibiotic resistance showed that the transformant *B. megaterium* was resistant to all antibiotics tested, except for ampicillin. In contrast, *A. chroococcum* was sensitive to gentamicin, cefotaxime, erythromycin, and streptomycin, while it resisted other antibiotics. Based on these results, erythromycin and ampicillin were selected as antibiotic markers for the conjugation process.

2.2. Conjugation between *A. chroococcum* and transformant *B. megaterium* with PCR of *nif* Genes:

Various species of bacteria frequently swap genetic material through a process known as conjugation, which occurs when cells come into contact with each other. This mechanism is significant for genetic modification research, horizontal gene transfer, and the spread of various types of antibiotic resistance, leading to extensive investigation into conjugation [76].

To study the transfer of *nif* genes (*nif*H1 and *nif*V) from *A. chroococcum* to transformant *B. megaterium*, a conjugation method was employed with *A. chroococcum* serving as the donor and *B. megaterium* as the recipient. Plates of Sperber agar supplemented with erythromycin and ampicillin yielded 170 transformant-conjugant colonies, indicating successful conjugation between the two species. To confirm the movement of the *nif* genes from the donor to the recipient, a molecular analysis was performed using PCR and gel electrophoresis. This analysis involved detecting the *nif*H1 and *nif*V genes with specific primers after extracting chromosomal DNA from the cells. Figures (5) and (6) show successful *nif*H1 (700 bp) and *nif*V (1146 bp) amplification from *A. chroococcum* donor cells Lane (2) and *B. megaterium* transformant-conjugant receiver cells Lane (4), respectively. The *B. megaterium* negative control Lane (3) yielded negative results in both figures. Previous studies have demonstrated *nif* gene transfer via conjugation: [77] transferred several *A. chroococcum nif* genes into *Klebsiella pneumoniae*, examining their expression in transconjugants; similarly, [78, 79] moved various *A. chroococcum* chromosomal *nif* genes into *Lactobacillus plantarum*. Additionally, [72, 73] transmitted some *A. chroococcum nif* genes into *B. megaterium* using this technique. Furthermore, [80] conjugated between *E. coli*-produced asparaginase and ZnO nanoparticles on cancer cell lines.

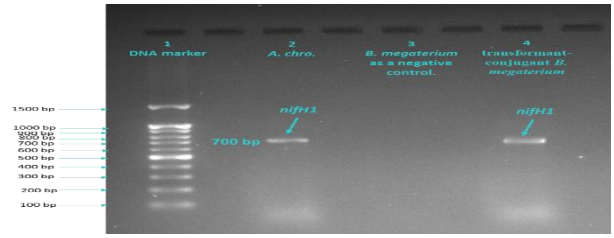

Figure 5. PCR results of the *nif*H1 gene with (700 bp):: DNA marker (lane1), *A. chroococcum* and transformant-conjugant *B. megaterium* are lane 2 and 4 respectively (+ve resuls), and lane 3 is *B. megaterium* (-ve result)

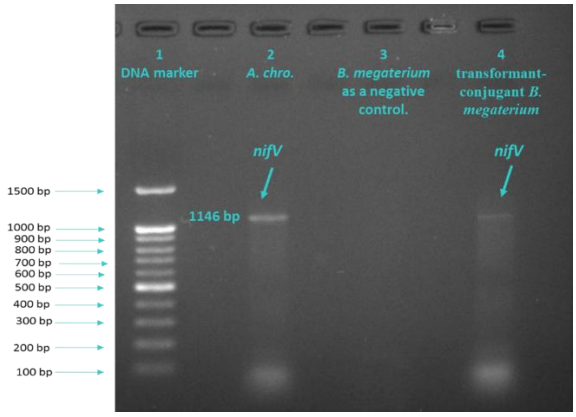

Figure 6. PCR results of the *nif*V gene with (1146 bp):: DNA marker (lane1), *A. chroococcum* and transformant-conjugant *B. megaterium* are lane 2 and 4 respectively (+ve resuls), and lane 3 is *B. megaterium* (-ve result)

Conclusions:

Colony morphology was studied for all isolated bacteria, and their presence was confirmed via PCR using species-specific primers after DNA extraction. *R. leguminosarum* harboured *nod*D1 and *nod*G genes, while *A. chroococcum* contained *nif*H1 and *nif*V genes. It was successfully transformed *B. megaterium* with *nod* genes from *R. leguminosarum* and conjugated *A. chroococcum* and *B. megaterium* to transfer *nif* genes.

Acknowledgments:

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التقنيات الفيزيوكيميائية والجزيئية لنقل nodD1، nodG من Rhizobium Leguminosarum؛ nifH1 و nifV من Azotobacter chroococcum الى Bacillus megaterium.

شهين كامل طالباني

الخلاصة

تضمنت هذه الدراسة استخدام تقنيتين لنقل بعض الجينات المهمة في تثبيت النيتروجين إلى *Bacillus megaterium*. في البداية تمت دراسة الخصائص المورفولوجية والجزيئية للتعرف على البكتيريا المعزولة. بعد عزل *Bacillus megateriu* و *Azotobacter chroococcum* و *Rhizobium Leguminosarum* باستخدام بيئات معينة لكل بكتريا، وقد أظهرت نتائج المورفولوجية بأن المستعمرات كانت دائرية الشكل ومحدبة وملساء لجميع أنواع البكتيريا،، وكان *A. chroococcum* ذو لون كريمي، في حين كان *B. megaterium* و *R.leguminsarum* أبيضا اللون. نجاح اسنخدام الطرق الجزيئية اعتمدت تم تحديد الحمض النووي الكروموسومي، و *nifH1* مع *nifV* لـ *A. chrococcum* ، والحمض النووي البلازميدي، و *nodD1* مع *nodG* لـ *R. Leguminosarum*، والحمض النووي الجينومي بالإضافة إلى اثنين من البادئات لـ *B. megaterium* على هلام الاغاروز. تم تحويل جينات *nod* من *R. leguminosarum*إلى *B. megaterium* عن طريق الحصول على 46 مستعمرة على اطباق أجار محتوية على بيئة سبيربر و المجتوية على التتراسيكلين والأمبيسيلين كدلائل وراثية بتقنية التحويل، وتم نقل جينات *nif* من *A. chroococcum* إلى *B. megaterium* المتحولة باستخدام آلية الاقتران، مما أدى إلى نمو 170 مستعمرة على اطباق أجار محتوية على بيئة سبيربر و المحتوية على الإرثيميسين بالإضافة إلى الأمبيسيلين كدلائل وراثية. وللتحقق من ذلك، تم إجراء تحليل جزيئي للكشف عن جينات *nod* في *B. megaterium* المتحولة وجينات *nif* في *B. megaterium* المتحولة - المقترنة باستخدام برايمرات المناسبة وشروط *PCR* لجميع الجينات مع الترحيل الكهربائي للهلام مما دل على أن التقنيتين قد اكتملتا بنجاح.

الكلمات المفتاحية: جينات تثبيت النيتروجين، التحول البكتيري ، الأقتران البكتيري, تقنية *PCR* ، الترحيل الكهربائي الهلامي.